ABSTRACT

Title of Document: CALORIC CONTENT OF BERING AND CHUKCHI SEA BENTHIC INVERTEBRATES

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Extensive seasonal sea ice reduction has highlighted the need to evaluate the status and potential long term changes of highly productive benthic communities in the Pacific Arctic Region. Walrus that use sea ice to access offshore feeding areas are now being forced to haul out on land for part of the year, requiring them to forage for benthic prey from closer to shore. To explore this energetic problem, I conducted a caloric survey of benthic invertebrates, and evaluated relationships between caloric content and environmental variables. Latitude was the strongest non-taxonomic dependency for caloric content (ANOVA $p=0.003$ with taxon dependencies, $p<0.001$ without). Cluster analysis revealed caloric densities were higher in offshore, high nutrient Bering Sea Anadyr Water, and lower in nearshore, low nutrient Alaska Coastal Water. An evaluation of preservation techniques indicated formalin fixation increased infaunal caloric content ($p<0.001$), suggesting caution while converting traditional benthic population studies to caloric values.

CALORIC CONTENT OF BERING AND CHUKCHI SEA BENTHIC INVERTEBRATES

By

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Chapter 1: Introduction

Research Interests in the Arctic

Arctic ecosystems have become the focus of increased research efforts in recent years due to changing environmental conditions and anthropogenic impacts related to climate change (IPCC 2007). The Intergovernmental Panel on Climate Change (IPCC) reports that winter warming of northern high latitude regions by the end of the 21st century will be at least 40% greater than the global mean, and that the areally averaged atmospheric warming of the Arctic is expected to reach 2-9°C by the year 2100 (IPCC 2001, 2007). High latitude sensitivity to climate change is likely related to a number of feedback mechanisms, including cloud radiation interactions (Liu et al. 2008; Leibowicz et al. 2012), methane release by melting permafrost (Zimov et al. 2006), and sea ice melt (Stroeve et al. 2011).

A marked decline in Arctic sea ice extent, an important driver of both climate and ecosystem change, has been observed since the first recorded satellite data was collected in the late 1970s (Figure 1.1) (Meier et al. 2007; Parkinson and Cavalieri 2008; Stroeve et al. 2011). Sea ice serves as a habitat for sea ice algae, and cycles of formation and melt fuel nutrient interactions that influence Arctic ecosystem structure, and play a major role in the formation of water masses (Grebmeier and Dunton 2000). Altered patterns of sea ice formation and melt and rising seawater temperatures have already been linked to observed changes in species ranges and compositions throughout the PAR (Grebmeier et

al. 2006; Grebmeier 2012), but there is a need to further evaluate these changes over long time scales.

In order to better understand the ecosystem response to physical changes occurring in the Arctic, the Pacific Arctic Group initiated the Distributed Biological Observatory (DBO) program in 2010, an international collaboration of field sampling and analyses at select marine sites in the Arctic. The primary goal of the DBO is to develop a change detection array along a latitudinal gradient in the Pacific Arctic Region (PAR), spanning from the northern Bering Sea to the Chukchi Sea, just off the coast of Barrow, Alaska. The DBO integrates environmental, chemical and biological studies for both the water column and the benthos in the PAR, and will link these data to observations of higher trophic level predators (Grebmeier 2012; see http://www.noaa.gov/dbo).

In conjunction with climate change, anthropogenic impacts are also intensifying in the Arctic, as northern waters are becoming more accessible for commercial oil and gas exploration (Harsem et al. 2011). A 2008 United States Geological Survey (USGS) report estimated reserves of oil and oil-equivalent natural gas in the Hope Basin (the region just north of the Bering Strait) to be approximately 122 million barrels, but the estimate for Arctic Alaska was almost 72,766 million barrels, the second highest level of the 33 provinces identified by the survey (Bird et al. 2008). In 2008, Shell Oil purchased approximately 34 million acres of the Chukchi Sea under Oil and Gas Lease Sale 193 (Figure 1.2) (USDOI MMS 2010).

In preparation for offshore oil and gas development, the U.S. Bureau of Ocean Energy Management (BOEM) supported a study called the Chukchi Sea Offshore Monitoring in Drilling Area (COMIDA) Chemical and Benthos (CAB) program to

undertake baseline environmental studies. The aim of this program included studies of benthic and epibenthic macroinvertebrates on the Chukchi continental shelf, along with water column and sediment nutrient and chemical analyses, sedimentation rates, and trace metals (see www.comidacab.org for further details).

This thesis project was developed to evaluate the current status of benthic and epibenthic invertebrate communities in the northern Bering and southern Chukchi Seas, under both the DBO and COMIDA CAB projects. Specifically, it aimed to evaluate the current (2010-2011) caloric values of benthic and epibenthic macroinvertebrate fauna, and analyze patterns in spatial distribution, explore explanatory environmental variables, and compare the effects of differing tissue preservation methods on caloric determinations.

Description of Study Area and Seasonal Ice Patterns

Quantitative samples for this study were collected between 62 °N, located in the northern Bering Sea just south of St. Lawrence Island, and 72 °N, located in the Chukchi Sea near Barrow, Alaska (Figure 1.3). All samples were collected in July – August 2010 and 2011.

The northern Bering Sea is a relatively shallow continental shelf, with depths <100 m in the study area (Stabeno et al. 1999). Current flow, which is influenced by wind and differences in sea level (Aagaard et al. 2006; Danielson et al. 2012), is generally northward moving through the 85 km wide Bering Strait (Coachman et al. 1975), with approximately 80% of its throughflow coming from the Anadyr Strait

(Danielson et al. 2012). High nutrient Anadyr water enters the northern Bering Sea from the western boundary of the Bering Sea Basin (Coachman et al. 1975; Schuert and Walsh 1993; Codispoti et al. 2005), whereas to the east, outflow from the Yukon River joins the northward flowing Alaska Coastal Current offshore of the Alaskan coast, forming a water mass low in nutrient content and productivity (Springer and McRoy 1993; Stabeno et al. 1999; Codispoti et al. 2005).

The Chukchi Sea is shallow, averaging approximately 50 m (Weingartner et al. 2005). As the dominant Anadyr and Alaska Coastal waters enter the southern Chukchi Sea through Bering Strait, they deliver heat, fresh water, and organic carbon from the Bering Sea (Weingartner et al. 2005). Moving northward, they diverge, with the more saline, high nutrient Anadyr water fanning across the central and northwestern portions of the Chukchi Sea, being modified to form Bering Sea water. By comparison, the fresher, low nutrient Alaska Coastal Water flows northward along the Alaskan Coast to the east (Coachman et al. 1975; Weingartner et al. 2005). These water masses also vary seasonally and annually due to melting and freezing of sea ice (Woodgate et al. 2005).

Maximum ice coverage in the Bering Sea in the past has occurred in February and March, with the ice-free period occurring from late June to late October (Mysak and Manak 1989). One of the most prominent features of the winter Bering Sea is the existence of a large cold pool near St. Lawrence Island (Schumacher et al. 1983), which in the past has supported benthic communities with some of the highest benthic biomasses and oxygen uptake rates in the northern Bering Sea, possibly due to the increased settling rates of organic matter as it is concentrated in the cold pool gyre (Grebmeier and Cooper 1995). In the southern Chukchi Sea, winter ice melts in the late

spring/early summer due to atmospheric circulation patterns and warm Bering Sea water entering the Chukchi Sea through the Bering Strait, although sea ice persists in the Herald and Hanna Shoal areas into late summer (Spall 2007).

Climate change forcing factors have led to changes in both atmospheric and oceanographic features. Ice cover over the Bering Sea shelf has significantly decreased between 1954 and 2006, and an increase in summer bottom temperatures has been linked to the retreat of the cold pool (Mueter and Litzow 2008). While decreases in ice over long time scales have been observed in the Bering Sea, it must be noted that interannual variability exists, with cold and warm periods lasting approximately 5 to 6 years (most recently ending with a cold period from 2007 to 2010) (Stabeno et al. 2012). In the Chukchi Sea, significant declines in summer sea ice extent (Serreze et al. 2007) and thickness (Kwok and Rothrock 2009) have been observed. This decline in sea ice has driven many local physical and biological changes, including altered water circulation patterns (Nghiem et al. 2007), warming of the mixed layer (Mathis et al. 2008), and increased light availability for primary production (Frey et al. 2011).

The Value of Caloric Surveys in Ecosystem Studies

Odum (1962) proposed that ecology could be separated into two branches of study: structure and function. From this perspective, structure can refer to community composition, the distribution of abiotic materials, and the gradient of environmental conditions, while function can refer to rates of material cycling, regulation by the physical environment and by organisms, and the flow of energy through ecosystems (Odum 1962). Ecologists have long understood the importance of studying energy flow

in ecosystems (Lindeman 1942). Because the abundance of individuals can overstate the importance of small organisms, and weight or biomass can overstate the importance of larger organisms, neither is useful alone for evaluating or comparing the functional roles of populations with different species compositions. By comparing the rates of energy flow in ecosystems, this problem can be overcome, and more informative and direct comparisons among communities can be made (Odum 1968). More recently, it has been proposed that evolutionary ecology is linked to ecosystem function, in the form of adaptive foraging (Schmitz et al. 2008).

Food web studies facilitate ecosystem understanding, as the number of energy transfers impact community structure, modify ecosystem function, and influence contaminant build up in higher trophic level predators (Post 2002). In the Arctic, food webs tend to be short with high densities of macroinvertebrates (Dunton et al. 1989; Iken et al. 2010; Grebmeier 2012). The biomass of these benthic communities is estimated to reach nearly 150 g C m^{-2} in some zones of the Pacific Arctic Region, with the highest biomass maintained in and around the Gulf of Anadyr and the central region of the Chirikov Basin in the Bering Sea, and at the head of Barrow Canyon in the Chukchi Sea (Figure 1.4) (Grebmeier 2012). Bivalves dominate southwest of St. Lawrence Island and in and around the southern portion of the Chukchi Sea, though dense communities of amphipods can be found in the central region of the Chirikov Basin (Figure 1.5) (Grebmeier 2012). By contrast, echinoderms can be also be found throughout the Chirikov Basin and southern Chukchi Sea, but are typically found closer to shore in the less nutrient rich waters of the ACW (Figure 1.5, 1.3) (Feder et al. 2005; Bluhm et al. 2009; Grebmeier 2012). Benthic communities, particularly molluscs, polychaetes and

amphipods, serve as a primary food source for a number of higher trophic level predators, including bottom feeding fish, whales, seals, walrus and diving birds (Fay et al. 1977; Lowry et al. 1980; Hazard and Lowry 1984; Highsmith and Coyle 1992; Lovvorn et al. 2003; Cui et al. 2009; Iken et al. 2010).

In the PAR, interest in marine mammal bioenergetics is increasing with changing environmental conditions (Geiselman et al. 2012). In order to evaluate the outlook for marine mammals in the PAR, food requirements and availability are key bioenergetic components that should be explored. When estimating the food requirements of marine mammals, it is necessary to evaluate both predator activity levels and the caloric content of the prey field (Kastelein et al. 2000). Caloric content, an indicator of food quality, also should be considered with food availability. Habitat zones that are characterized by high prey density and/or high caloric density are known to be preferred feeding grounds for Arctic marine mammals (Bluhm and Gradinger 2008). Nutritional stress due to poor prey quality is considered to be a major cause of observed declines in Alaskan Steller sea lions (*Eumetopias jubatus*) in areas to the south of PAR, emphasizing the importance of studying prey quality (Trites and Donnelly 2003).

Rationale for Study

Over the past 40 years, caloric energy studies in temperate ecosystems have been common, but recently an increasing number of caloric studies have been undertaken in the Arctic. In particular, benthic invertebrate caloric energy surveys have been conducted in the European Arctic (Szaniawska and Wolowicz 1986; Weslawski et al. 2010), northern Atlantic, and Atlantic Arctic (Tyler 1973; Wacasey and Atkinson 1987), but few

caloric studies have occurred in the PAR. A mid to late 1970s effort reported formalin-preserved caloric values for 52 species of benthic infauna within 5 classes, and found organic carbon and caloric content to be highly correlated (Stoker 1978). In that study, formalin-preserved caloric densities averaged 4.85±0.13 kcal/g for bivalves, 3.60±0.76 kcal/g for polychaetes, and 5.22±0.24 kcal/g for amphipods (Stoker 1978). A limited comparison of caloric content between formalin-preserved and frozen animals was conducted as part of this investigation and included nine bivalve taxa (average of 4.85±0.14 kcal/g formalin-preserved versus 4.42±0.22 kcal/g frozen), and 6 other miscellaneous taxa (overall average of (4.53±0.30kcal/g formalin-preserved versus 4.14±0.30 kcal/g frozen). Miscellaneous taxa included two polychaete worms (F. Maldanidae and *Nephtys sp*.) (3.66±0.32 kcal/g formalin-preserved versus 3.64±0.48 kcal/g frozen) and one species of amphipod (*R. Aculeata*) (3.96 kcal/g formalin-preserved versus 4.29 kcal/g frozen) (Stoker 1978).

A recent study by Hondolero et al. (2011) evaluated the caloric content of a subset of PAR faunal organisms, covering 18 epifaunal taxa and 6 infaunal taxa. Reported values for formalin-preserved benthic invertebrates from that study ranged from 2.45-5.00 kcal/g. While variable results suggested that preservation method (formalin-preserved versus frozen) plays a role in varying caloric values, low sample size may have obscured significant differences. In addition, the low number of species sampled may prevent making larger connections between the prey field caloric measurements and higher trophic level predators.

This thesis project was developed to evaluate caloric values for benthic macroinvertebrates on a wide spatial scale throughout the PAR, and to investigate the

relationships between faunal caloric content and environmental variables in the PAR. Another goal of the thesis project was to investigate how preservation method influences faunal caloric content in order to evaluate the potential for comparative studies with caloric content of fauna obtained using various methodologies in the past.

Statement of Hypotheses and Thesis Structure

Seven testable hypotheses were formulated to approach the problems outlined in the previous section:

Hypothesis 2.1: There is no significant difference in caloric content by taxonomic type in the Chukchi Sea study area.

Alternative Hypothesis: Taxonomic differences in caloric values exist among faunal types.

Hypothesis 2.2: There is no significant difference in infaunal caloric content among stations in the Chukchi Sea study area with varying sediment organic carbon and nitrogen content, which are used as indicators of food quality and quantity.

Alternative Hypothesis: Caloric content is high in areas with high organic carbon and nitrogen content, which are used as indicators of high food quality and quantity.

Hypothesis 2.3: There is no latitudinal difference in benthic faunal caloric content moving northward in the 2010 Chukchi Sea study area.

Alternative Hypothesis: Caloric content in benthic fauna increases northward within the 2010 Chukchi Sea study area.

Hypothesis 2.4: There is no significant difference in taxon caloric content among benthic fauna living in different water masses in the Chukchi Sea study area.

Alternative Hypothesis: Since productivity is higher in offshore Anadyr Water in the Chukchi Sea study region, and there is likely to be more pelagic-benthic coupling, there are higher caloric densities among benthic fauna living in northwestern Anadyr water compared to the less productive Alaska Coastal water.

Hypothesis 3.1: There is no significant difference among organisms in the same taxon preserved by freezing or formalin.

Alternative Hypothesis: Because of differences in lipid content, significant differences in caloric content exist among bivalves, amphipods and polychaetes.

Hypothesis 3.2: Caloric content of benthic infauna collected from the Chukchi Sea will not significantly differ from benthic macroinvertebrates collected in the northern Bering Sea.

Alternative Hypothesis: Significant differences in the caloric content of benthic macroinvertebrates exist between the northern Bering and Chukchi Seas.

Hypothesis 3.3: There is no significant difference between the caloric values of benthic infaunal tissues preserved in formalin versus frozen samples.

Alternative Hypothesis: Formalin-preserved samples will have significantly higher caloric densities than frozen infaunal samples because formalin preservation adds carbon to tissues.

The above hypotheses are addressed in two subsequent chapters. In Chapter 2, I model variance in caloric content throughout the Chukchi Sea COMIDA CAB study area. Current caloric energy values of benthic and epibenthic macroinvertebrates in the Chukchi Sea are reported, and the values analyzed for relationships to various spatial and environmental variables. Models are then created to determine which spatial and environmental variables are most significantly dependent on macroinvertebrate caloric content. Two versions of the linear model are presented, one with the influence of taxon included and the other with the influence of taxon regressed out. A mixed effects model is also presented, and 3 approaches to cluster analysis (2 partitioning and 1 hierarchical agglomerative) are included in the analysis. These findings have implications for Pacific walrus (*Odobenus rosmarus divergens*) foraging patterns in particular because they are major consumers of benthic macroinvertebrates in the PAR. Changing seasonal sea ice patterns may be impacting their access to preferred foraging areas from overlying sea ice that is now retreating off the continental shelf (Jay et al. 2011).

In Chapter 3, a methodological investigation is presented. The effects of two preservation methods (formalin fixation and freezing) on the apparent caloric energy content of prey organisms are compared. A major goal of this study was to generate conversion factors between wet weight biomass and kilocalories. These results will facilitate the conversion of past wet weight biomass data in the PAR to energetic terms, leading to broader spatial and temporal scale comparisons of caloric prey content. This method could be very informative for predator-prey studies.

Chapter 4 provides a summary and conclusion of the thesis effort.

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Figures

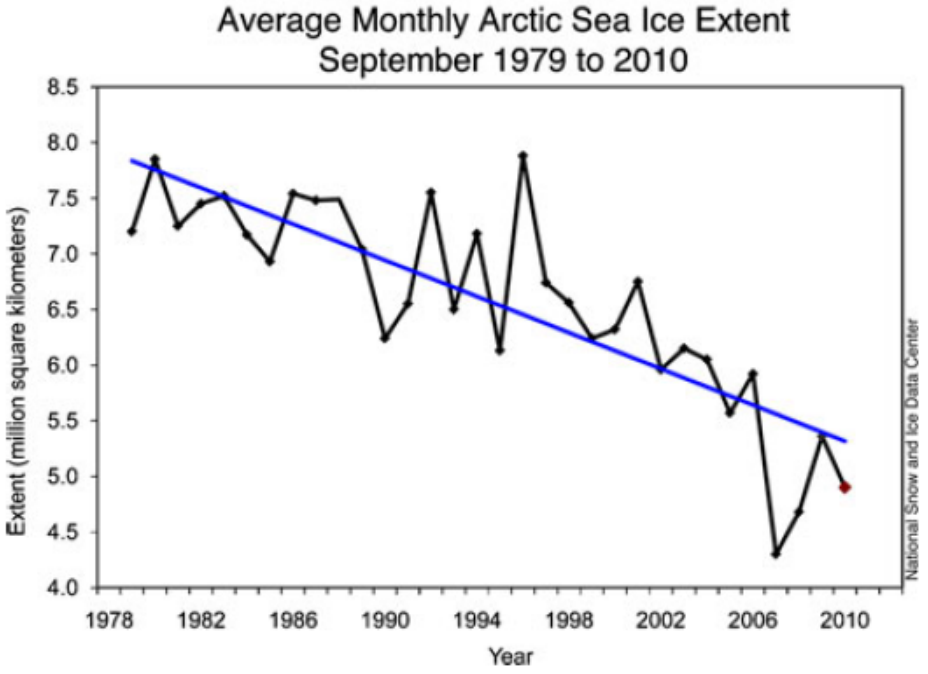

Figure 1.1: Time series of average monthly arctic sea ice extent from September 1979 to 2010 (from Stroeve et al. 2011).

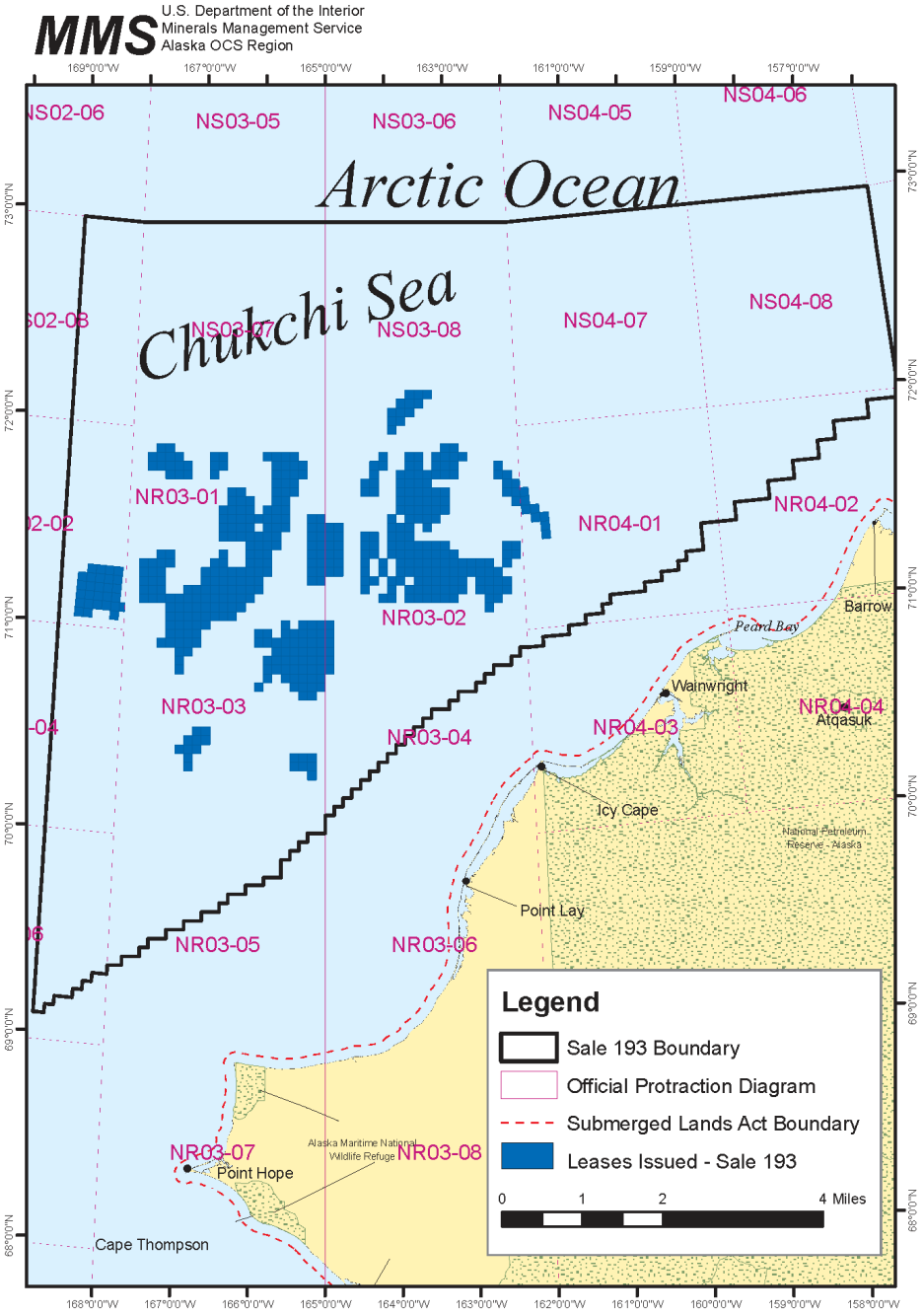

Figure 1.2: Map of Oil and Gas Lease Sale 193 in the northern Chukchi Sea (USDOI MMS 2010).

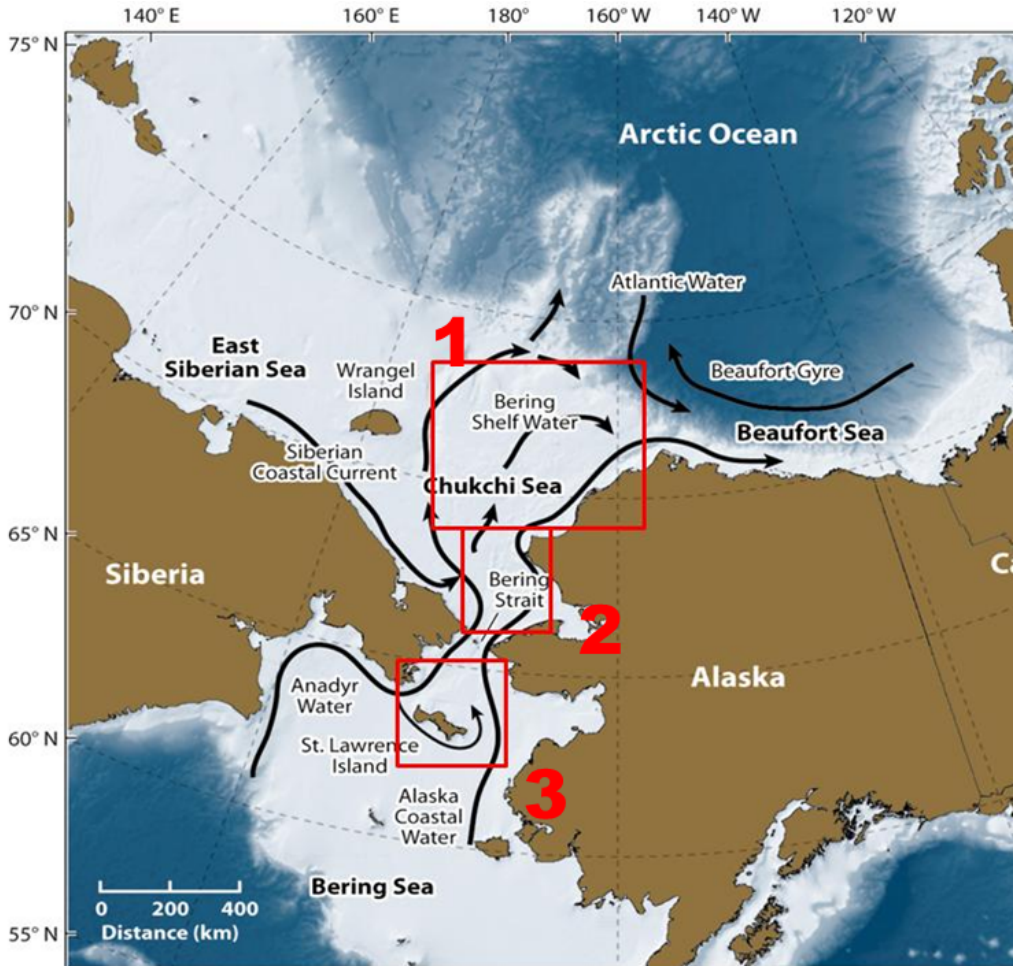

Figure 1.3: Map of the Pacific Arctic Region (PAR), with water masses and circulation patterns (modified from Grebmeier 2012). Areas surveyed in this study are indicated by red boxes. Box 1 represents a zone sampled during the Chukchi Sea Offshore Monitoring in Drilling Area (COMIDA) Chemical and Benthos (CAB) cruise in July-August 2010 and the Distributed Biological Observatory (DBO) cruise in July 2011, and boxes 2-3 represent zones sampled during the DBO cruise in July 2011.

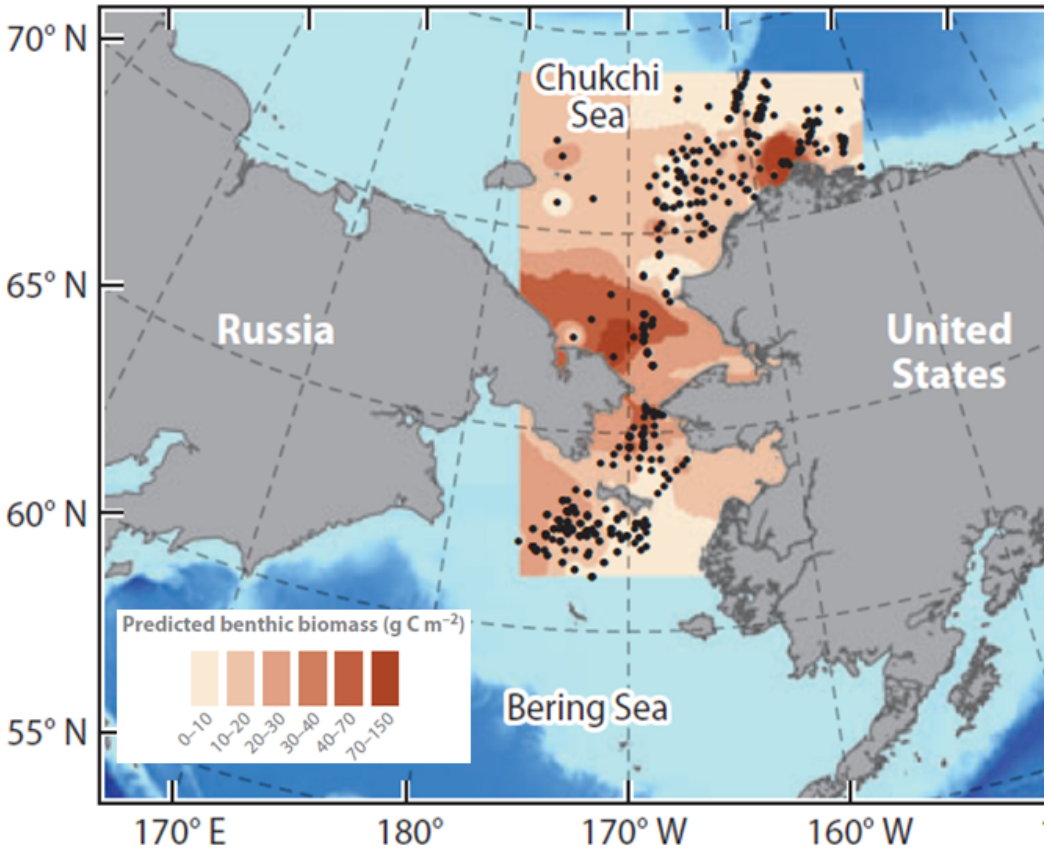

Figure 1.4: Distribution of infaunal benthic biomass in the Pacific Arctic Region, 2000-2010 (modified from Grebmeier 2012). Black dots signify station locations.

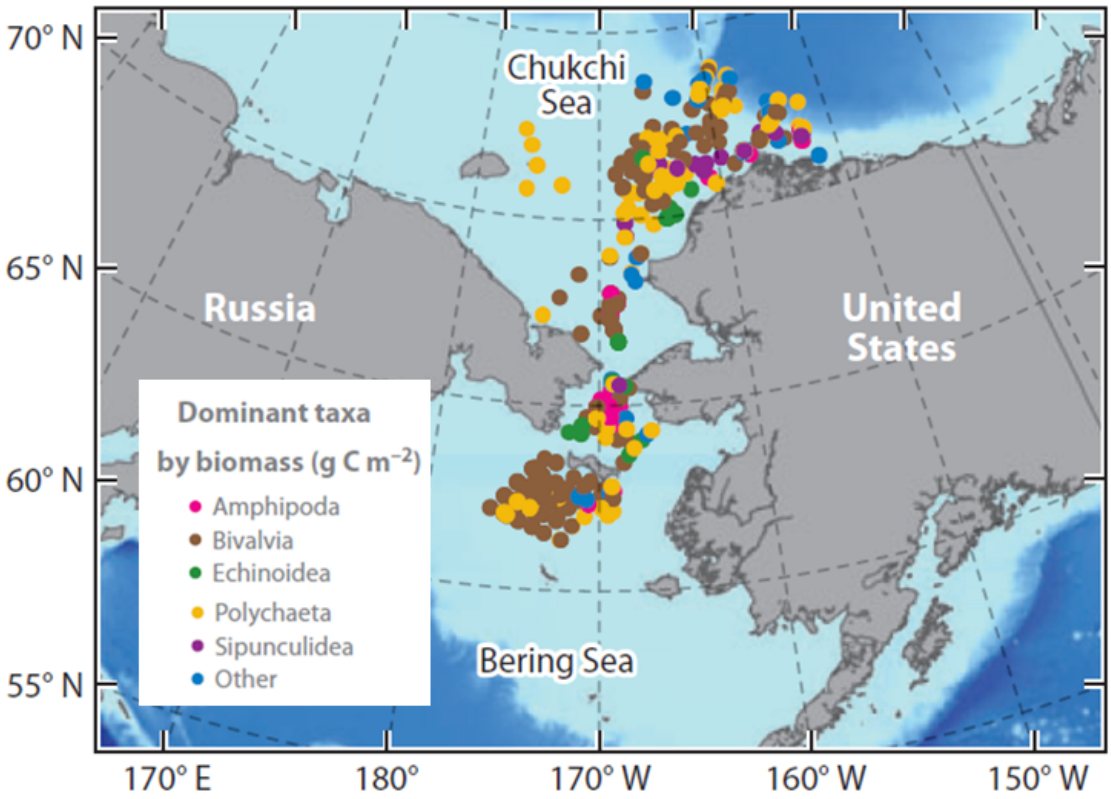

Figure 1.5: Distribution of dominant infaunal groups in the Pacific Arctic Region, 2000-2010 (modified from Grebmeier 2012). Each dot signifies a station, color coded by dominant taxon.

Chapter 2: Modeling Variance in Caloric Content Throughout the Chukchi Sea Study Area

Abstract

The Chukchi Sea shelf off the northern coast of Alaska is rich with benthic and epibenthic macroinvertebrates that support Pacific walrus (*Odobenus rosmarus divergens)* and other benthic-feeding higher-level consumers. Recent sea ice retreat on the Chukchi Sea shelf has led to walrus haul-outs on beaches of the Chukchi Sea in Russia and Alaska. The need to fully assess the impacts of foraging from shore led to the current study, which constrains walrus food supply with energetic requirements. 171 caloric values were obtained for 11 classes of benthic fauna over 15 southeastern Chukchi Sea stations in 2010. With α set at 0.05, Spearman correlation statistics indicated significant relationships between caloric content and latitude ($R=0.661$) and bottom temperature ($R=-0.560$). In addition, Pearson correlation statistics indicated significant relationships between caloric content and grain size (% sand $r=-0.562$, % silt $r=0.541$), and sediment total organic nitrogen ($r=0.574$). Linear modeling indicates that taxon and latitude are the greatest dependencies for caloric content, whereas a second model with taxon dependencies removed returned significant coefficients for the explanatory variables of latitude, depth, bottom water temperature, sediment total organic carbon, and sediment total organic nitrogen. K-means cluster analysis produced 6 clusters with 86% variance between clusters. Clustering was based mainly upon environmental variables such as bottom temperature, bottom salinity, and other local measurements. The characteristics of the observed clusters were clearly distinguished by their caloric content and geographical location, particularly latitude. The finding that caloric content varies so strongly with latitude, a proxy for both water mass type and coincident water mass productivity in the study area, may have implications for Pacific walrus, whose typical foraging patterns offshore have been disrupted by sea ice decline.

Introduction

Research Interest in the Chukchi Sea

The Arctic has become a prime location for studying climate change effects on ecosystems, as marked declines in sea ice, an important driver of both climate and ecosystem change, have been observed since the first recorded satellite data in the late 1970s (Meier et al. 2007; Parkinson and Cavaliei 2008). In particular, significant declines in summer ice extent (Serreze et al. 2007) and thickness (Kwok and Rothrock 2009) have been noted in the Chukchi Sea (Codispoti et al. 2005). This has driven many local physical and biological changes, including altered water circulation patterns (Nghiem et al. 2007), warming of the mixed layer (Mathis et al. 2008), and increased light availability for primary production (Frey et al. 2011).

Commercial oil and gas interests and a need to evaluate benthic prey populations in relation to higher trophic level foraging use in the planned exploration area have highlighted the need for larger spatial and temporal scale ecosystem studies in the Chukchi Sea, leading to the development of the BOEM (Bureau of Ocean Energy Management) Chukchi Sea Offshore Monitoring in Drilling Area (Chemical and Benthos) (COMIDA CAB) program. The aims of this program include the development of a baseline dataset for the benthic and epibenthic macroinvertebrates that dominate the Chukchi Sea continental shelf, sea water chemistry, physical parameters, and sediment characteristics, including sedimentation rates and trace metal content (see www.comidacab.org).

Chukchi Sea Ecosystems: Linkages between the Water Column and Benthos

Biological processes in the Chukchi Sea have strong inherent variability by season, similar to other regions of the Arctic Ocean (Grebmeier et al. 1995). The Chukchi Sea supports one of the highest levels of marine ecosystem productivity in the world (Hill and Cota 2005; Grebmeier et al. 2006a; Bluhm and Gradinger 2008; Gradinger 2009) due to sea ice melt, the movement of nutrient rich water masses north through the Bering Strait (Coachman 1987, Weingartner et al. 2005), and tight benthic-pelagic coupling of upper water column organic carbon production settling to the underlying shallow continental shelf (Grebmeier et al. 1988; Campbell et al. 2009; Iken et al. 2010). In the past, estimates of annual primary production in the Chukchi Sea have surpassed 250 g C m^{-2} d^{-1} (Walsh et al. 1989). More recently, spring rates of primary production in the ice covered Chukchi Sea measured <0.3 g C m^{-2} d^{-1}, but reached 8 g C m^{-2} d^{-1} during ice break up (Hill and Cota 2005).

Within the Chukchi Sea, a number of water masses have been identified and studied with relevance to benthic communities, including the high nutrient Anadyr Water (AW) entering the southern Chukchi Sea from the western side of Bering Strait, the low nutrient Alaska Coastal Water (ACW) entering the southern Chukchi Sea from the eastern side of Bering Strait, and the mixed Bering Shelf Water (BSW) in between these two water masses (Coachman 1987; McRoy 1993; Weingartner et al. 2005). Though the ACW water mass remains distinct from the other two water masses as it moves northward along the Alaska Coast into the Chukchi Sea, portions the AW and BSW water masses mix as they move north and westward. This merged water mass has previously been designated as Bering Shelf-Anadyr Water (BSAW), or more recently the Bering Sea

water (winter vs summer) in the central and northern Chukchi Sea (Weingartner et al. 2005), which is known to have a much higher quality carbon supply to the benthos than the ACW in summer (Grebmeier et al. 1988). Higher nutrient supply in BSAW supports greater overall annual primary and secondary production than in the ACW water mass (Stoker 1978; Walsh et al. 1988). Annual primary production in the ACW water mass is characteristically low (20-70 g C m^{-2} y^{-1}), whereas the annual primary production rate in Anadyr waters tends to be high (470 g C m^{-2} y^{-1}) (Springer et al. 1996; Sakshaug 2004; Grebmeier et al. 2006a).

In the northern Bering and Chukchi Seas, benthic infaunal biomass is estimated to reach nearly 150 g C m^{-2}, with the highest biomasses found in the Gulf of Anadyr, southwest of St. Lawrence Island, in the southern Chukchi Sea, and at the head of Barrow Canyon (Grebmeier 2012). Specifically for BSAW, a high benthic faunal abundance of 13,554 ind m^{-2} has been observed, with carbon biomass ranging from 0.3 to 56.2 g C m^{-2} (Feder et al. 2007). In the last decade, bivalves (F. Tellinidae and Nuculanidae), sipunculids (F. Golfingiidae), amphipods (F. Ampeliscidae and Lysianassidae), isopods (F. Idoteidae) and polychaetes (F. Maldanidae and Nephtyidae) have dominated the biomass in the Chukchi Sea, though assemblages of other organisms such as sea anemones, gastropods and sand dollars have also been observed (Grebmeier 2012). The region with the highest known benthic biomass in the Chukchi Sea (composed mostly of mussels and sipunculids) is in upper Barrow Canyon off the northern coast of Alaska, which is thought to be due to organic carbon delivery from the southern Chukchi Sea and high local primary production following ice melt in the spring (Grebmeier 2012).

Epibenthic invertebrate communities in the Chukchi Sea have an estimated gross abundance range of 229 to 70,879 individuals 1000 m^{-2} and a biomass estimate range of 1,628 – 21,7023 g wet weight 1000 m^{-2}, with high proportions of echinoderms, crustaceans and molluscs (Bluhm et al. 2009). While molluscs in the southeastern Chukchi Sea are highly diverse, echinoderms dominate by biomass, representing 59.7% of epifaunal biomass (Feder et al. 2005). While infaunal molluscs tend to cluster by abundance with the percentage of sand and bottom salinity, epifaunal molluscan groups tend to be clustered by percent gravel and bottom temperature (Feder et al. 1994). Food availability in the form of entrained suspendended sediment particulate organic carbon (POC) is also noted as a key driver of molluscan epifaunal abundance (Feder et al. 1994).

Food Web Links to Pacific Walrus and other Higher Trophic Level Predators

Food webs in the Chukchi Sea tend do not statistically differ in length between the higher nutrient AW water mass and the lower nutrient ACW water mass, but higher proportions of consumers in the first trophic level in AW indicate a more direct coupling of benthic macroinvertebrates to pelagic primary producers than in the ACW water mass (Iken et al. 2010). In keeping with these short food web lengths, benthic macroinvertebrates are key sources of food for higher trophic level predators such as bottom feeding fish, whales, seals, and diving birds (Lowry et al. 1980; Hazard and Lowry 1984; Highsmith and Coyle 1992; Lovvorn et al. 2003; Cui et al. 2009; Iken et al. 2010). In particular, Pacific walrus (*Odobenus rosmarus divergens*) are major consumers of benthic fauna. It has been estimated that the walrus population consumes

approximately 3 million tons of benthic biomass per year spanning over thousands of square kilometers in the Bering and Chukchi Seas (Fay 1982; Ray et al. 2006).

Pacific walrus have established annual cycles of movement throughout the Bering and Chukchi Seas, with adult females and calves following the receding ice pack northward into the Chukchi Sea in the spring, while adult males remain in the Bering Sea to the south. As winter ice develops in the Chukchi Sea in the autumn, adult females and young then return to the Bering Sea (Fay 1982). Pacific walrus depend upon the availability of dense populations of benthic invertebrates in shallow (<100 m) water, with nearby ice or land to haul-out on over their feeding grounds (Fay 1982). They also utilize ice in the marginal ice zone as transport and resting platforms for feeding grounds that are too far from shore (Kovacs et al. 2010). Pacific walrus prey upon a wide variety of benthic invertebrates, but prefer softer bodied organisms that are high in fat content (Fay 1977; Fay 1982; Sheffield and Grebmeier 2009). In particular, stomach content surveys have concluded that bivalves, gastropods and polychaete worms are the most frequently consumed prey items by Pacific walrus (Sheffield and Grebmeier 2009).

Because walrus have a low rate of reproduction, populations tend to respond negatively to environmental changes (Fay 1982). This factor may become problematic for Pacific walrus populations in the Chukchi Sea, as major environmental changes associated with climate change are underway. Receding sea ice, which is expected to limit access to feeding areas, is particularly problematic (Rausch et al. 2007, Jay et al. 2011). Declining Arctic sea ice has led to massive haul-outs in northwestern Alaska and along the Russian Arctic coast in the summer and fall of 2007 and 2009 (Jay et al. 2011), and many young walruses have been trampled at haul out sites (Fischbach et al. 2009).

Despite increases in haul-out behavior, numerous walrus have also been observed via satellite radio-tags making seemingly costly energetic swims far out to the north from land-based haul-out sites to reach preferred feeding grounds (Jay et al. in prep.).

In addition to limited access to sea ice, there are also increasing concerns for the Pacific walrus population due to changing prey availability and quality (Jay et al. 2011), as declining sea ice and increasing water temperatures may result in increased pelagic consumption and decreased benthic production (Grebmeier et al. 2006b). Ocean acidification may additionally pose problems for calcium dependent bivalves and gastropods (Guinotte and Fabry 2008), a favorite prey for walrus. For another Alaskan pinniped, the Stellar Sea Lion (*Eumetopias jubatus*), that is found south of Bering Strait, it is known that population declines are, at least in part, related to the quality of prey items (Trites and Donnelly 2003), but these types of connections have not yet been confirmed for Pacific walrus.

Caloric Surveys in the Chukchi Sea

While numerous caloric surveys have been conducted throughout the European and Canadian Arctic (e.g. Tyler 1973; Szaniawska and Wolowicz 1986; Wacasey and Atkinson 1987; Percy and Fife 1980), few have been conducted in the Chukchi Sea. In the mid to late 1970s, one of the most comprehensive caloric surveys conducted in the Bering and Chukchi Seas reported formalin-preserved caloric values for 52 species of benthic infauna encompassing 5 taxonomic classes (Stoker, 1978). In this survey, formalin-preserved caloric contents averaged 4.85±0.13 kcal/g for bivalves, 3.60±0.76

kcal/g for polychaetes, and 5.22±0.24 kcal/g for amphipods. Significant correlations were found to exist between organic carbon and caloric content, but analysis for the influence of other spatial and environmental variation on caloric content were not undertaken (Stoker 1978). A more recent survey by Hondolero et al. (2011) in the Bering and Chukchi Seas evaluated the caloric content of 18 epifaunal taxa and 6 infaunal taxa, including bivalves, polychaetes and crustaceans. The aim of that study was to compare the apparent caloric contents of benthic macroinvertebrates prepared under various preservation methods, as opposed to evaluating variation in caloric content with spatial location and environmental parameters. Reported caloric densities for formalin-preserved benthic invertebrates from that study ranged from 2.45-5.00 kcal/g, and for frozen benthic invertebrates values ranged from 2.45-4.77 kcal/g (Hondolero et al. 2011). In three out of the seven invertebrate taxa surveyed for caloric content, significant differences between formalin-preserved specimens and frozen specimens were observed, including a decapod (*Argis lar*) ($p=0.013$), and two anthozoans ($p=0.046$ and 0.050), but conclusions were limited by the small sample size (Hondolero et al. 2011).

While few studies have been conducted to specifically analyze explanatory environmental variables for caloric content, it has been documented that differences in energy can exist among taxa. Bivalves and amphipods have consistently higher caloric densities than polychaetes and echinoderms, though seasonality and geographic location can introduce significant variation in lipid content and thus caloric content, which likely explains the variability in study results (Stoker 1978; Wacasey and Atkinson 1987; Parrish et al. 1996; Gallagher et al. 1998; Hondolero et al. 2011).

Objectives of Study

The primary objective of this study was to determine the current caloric energy values of benthic and epibenthic macroinvertebrates in the Chukchi Sea. These values were then analyzed for relationships to various spatial and environmental variables. Statistical modeling approaches were used to determine which spatial and environmental variables are significant dependencies for macroinvertebrate caloric content, both with and without the influence of taxon. Cluster groups for determining similarity in caloric content and other environmental characteristics were also generated. The spatial layout of caloric density throughout the Chukchi Sea study area is of particular interest for understanding higher trophic level impacts on predators such as walrus.

Statement of Hypotheses

Hypothesis 2.1: There is no significant difference in caloric content by taxonomic type in the Chukchi Sea study area.

Alternative Hypothesis: Taxonomic differences in caloric values exist among faunal types.

Hypothesis 2.2: There is no significant difference in infaunal caloric content among stations in the Chukchi Sea study area with varying sediment organic carbon and nitrogen content, which are used as indicators of food quality and quantity.

Alternative Hypothesis: Caloric content is high in areas with high organic carbon and nitrogen content, which are used as indicators of high food quality and quantity.

Hypothesis 2.3: There is no latitudinal difference in benthic faunal caloric content moving northward in the 2010 Chukchi Sea study area.

Alternative Hypothesis: Caloric content in benthic fauna increases northward within the 2010 Chukchi Sea study area.

Hypothesis 2.4: There is no significant difference in taxon caloric content among benthic fauna living in different water masses in the Chukchi Sea study area.

Alternative Hypothesis: Since productivity is higher in offshore Anadyr Water in the Chukchi Sea study region, and there is likely to be more pelagic-benthic coupling, there are higher caloric densities among benthic fauna living in northwestern Anadyr water compared to the less productive Alaska Coastal water.

Methods

Sample Collection and Preparation

Samples were collected at 45 stations between July 25th and August 16th, 2010 from the RV Moana Wave in the Chukchi Sea as part of the COMIDA CAB project. Stations for the core COMIDA CAB project were selected in 2009, using both a general randomized tessellation stratified design (GRTS) in the core study area and a spatially-oriented, nearshore to offshore, south to north grid overlaying the stratified design. In addition, during 2010, stations were also sampled in Bering Strait and other regions in the Chukchi Sea to complete the spatial grid. For the current caloric project, 15 stations were selected for caloric analyses at random within three zones (nearshore, midshore, and

offshore), and four quadrants (northeast, northwest, southeast, and southwest) (Table 2.1; Figure 2.1).

Animals were collected using a weighted 0.1 m^2 van Veen grab for infaunal collections and a 3 m beam trawl for epibenthic faunal collections. Sediments were sieved through 1 mm screen mesh, and the retained samples collected for on-ship analyses. Infaunal samples for caloric content analyses were sorted shipboard to the lowest taxon possible (typically family), and frozen for preservation and transport to Chesapeake Biological Laboratory (CBL) in Solomons, MD, where they were stored in a -20°C freezer prior to processing for caloric analyses.

Animals were prepared for caloric analyses by first removing all non-organic materials, including the calcium carbonate shells of all bivalves and gastropods, and polychaete sediment tubes, following the methodologies of Wacasey and Atkinson (1987) since only the soft portion of the animal is typically be consumed by a feeding walrus (Fay 1982). Crustaceans and echinoderms were processed whole, though brittle stars (F. Ophiuridae) were omitted because the high amounts of dry skeletal material prevented accurate combustion with the instruments available, a problem that has also been noted by other investigators (Stoker 1978).

All processed samples were then weighed before placement in aluminum tins for desiccation in an oven at 80°C. Samples were weighed periodically over the following days until constant weight was achieved (typically in 5 to 6 days), indicating that the state of total desiccation necessary for calorimetry was achieved. A mechanical grinder was used to mix the dried tissues into homogeneous powders, which were stored in glass desiccators containing DRIERITE® until subsequent analyses. A pellet press (Parr

Instruments, Moline IL) was used to create either 1-3 g pellets or 0.1-0.5 g pellets, depending on the amount of sample available. For samples too dry for pelletization, gel capsules were used. Pellet weights and gel capsule weights were recorded for each sample prior to calorimetry.

Calorimetry Procedures

All pellets were combusted in a Bomb Calorimeter (Model 6200, Parr Instruments, Moline, IL). A large bomb (Model 1108, Parr Instruments) was used to analyze 1-3 g pellets, and a semi-micro bomb (Model 1109, Parr Instruments) was used for 0.1-0.5 g pellets. Caloric density estimates for both bombs were calibrated using a 1 g or 0.1 g benzoic acid (C6H5COOH) pellet. Caloric densities were measured in megajoules per kilogram (MJ/kg), and were corrected for the amount of fuse wire consumed in combustion and the remaining sample weight. Samples combusted in gel capsules were further corrected according to the formula:

$$E_c = ((E_s * w_p) - (E_{gc} * w_{gc}))/(w_p - w_{gc})$$

where:

E_c = corrected energy density

E_s = energy density of pellet containing sample

w_p = weight of the pellet containing sample

E_{gc} = energy density of the empty gel capsule

w_{gc} = weight of the empty gel capsule

For this capsule correction, five gel capsules were weighed to calculate an average gel capsule weight, and combusted for the calculation of an average gel capsule caloric density. The average weight was found to be 0.115±<0.01 g and the average caloric density was measured as 19.51±0.09 MJ/kg. These values were used in the formula to calculate the corrected caloric densities. Replicate tissue samples were burnt until the 2% difference level between values was reached, at which point the replicates were averaged.

Statistical Analysis

All statistical analyses were undertaken using R (version 2.15.0, see http://www.r-project.org). For a full listing of R packages and versions see Appendix 1.

1. Descriptive Statistics and Differences between Classes

Basic statistics for all stations and classes were calculated, including a) the mean caloric content over the entire Chukchi Sea study area, b) mean caloric content, variance and standard deviation for each station, and c) mean caloric content, variance and standard deviation for each class that was observed in the study. Because normality testing could not be carried out for the full dataset (2 classes had only 1 caloric observation), a non-parametric Kruskal-Wallis rank sum test was applied to evaluate if significant differences in caloric content exist by class.

2. Correlation Analysis

Relationships between average measured caloric densities at each station and the following parameters were evaluated: latitude, longitude, depth, bottom temperature, bottom salinity, sediment chlorophyll *a*, grain size, modal size, sediment total organic carbon (TOC), sediment total organic nitrogen (TON), and carbon to nitrogen ratio (C:N) (Table 2.2). All environmental data were obtained through the core COMIDA CAB program (COMIDA CAB 2012 final report available for download at http://www.comidacab.org/). A non-parametric Spearman's Rank correlation was conducted, and a parametric Pearson's correlation was also used for those variables that satisfied normality testing by Anderson Darling test. A matrix of pie charts representing r values was generated to illustrate cross-correlations among variables.

3. Analysis of Variance

Analysis of variance (ANOVA) was employed to model caloric content as a function of the explanatory variables. In this section of the investigation, zone, quadrant, sediment grain size, and sediment carbon to nitrogen ratio (C/N) were dropped from analysis because of strong interrelation with other variables. For example, latitude and longitude are superior descriptors to use for stations than station number, or zone and quadrant which are arbitrary. Because sediment chlorophyll a is so highly correlated with grain size, grain sizes were dropped from the analysis to avoid an overfit model. C:N ratio was also dropped for the inclusion of sediment TOC and TON. Adjusted r^2 and

Mallow's C_p were used to calculate and plot the number and combination of variables that would comprise the best fit for two different linear models, both explaining caloric content in the Chukchi Sea study area. The first model was created on a dataset that still included the influence of taxon (class) on caloric content. Because the variable of "lowest taxon identified" consumed too many degrees of freedom, it was dropped from this part of the analysis.

The second linear model was created after the dependencies on the two taxonomic variables (class and lowest taxon identified) were regressed out, so that the residuals comprised the new response variable. Since the remaining variables after regressing caloric content on class and "lowest taxon identified" were all associated with the station where the data was taken, the residuals were averaged by station before performing further regression analysis on the residuals. For both linear models, Tukey Honest Significant Differences (HSD) tests were applied to investigate differences between factor levels. Diagnostic plots were generated to evaluate the assumptions associated with ANOVA. Following these two ANOVA analyses, a nested mixed effects analysis was also conducted for this dataset. Class, and lowest taxon identified within class were identified as random effects, and latitude, longitude, sediment chlorophyll *a*, bottom salinity, and bottom temperature were identified as fixed effects.

4. Cluster Analysis

Sampling stations were clustered by ignoring categorical variables and using the average of the numerical variables caloric content, depth, bottom temperature, bottom salinity, sediment chlorophyll *a*, sediment grain size, sediment modal size, sediment

TOC, sediment TON, and carbon to nitrogen ratio (C:N) for each station. Since the variables have different means and standard deviations, prior to cluster analysis, all variables were scaled to a mean of zero and a standard deviation of 1. Two versions of partitioned clustering were applied to the numeric factors in the averaged dataset: K-means and the most robust Partitioning Around Medioids (PAM). Because a number of environmental observations were not completed for station 21 (CBL6), station 21 was excluded from cluster analysis. For K-means clustering, the recommended number of cluster groups was identified using a plot of the within groups sum of squares against clusters extracted. For PAM clustering, the optimal number of cluster groups was found by generating of a plot of average silhouette width (ASW), a measure of how far apart clusters are compared to their width bounded by -1 (intermixed) and 1 (separated), plotted against the number of clusters extracted (the cluster with the highest ASW was used for PAM cluster analysis). The Ward method of clustering was also employed as a second approach to cluster analysis (hierarchical agglomerative), using the number of clusters the ASW plot suggested based on maximum ASW.

Results

Stations and Classes

A total of 171 caloric values were determined for 11 classes of infaunal and epifaunal macroinvertebrates across all 15 stations throughout the Chukchi Sea study area (Appendix 2). The number of energy observations at each station ranged from 6 to 18,

and the number of classes represented at each station ranged from 4 (station #4, name=RDM) to 8 (station #20, name=1014). The overall mean energy content was 19.71±2.08 MJ/kg, ranging from a low 13.70 MJ/kg for a tunicate (F. Styelidae) to a high 23.49 MJ/kg for a bivalve (F. Nuculanidae). Mean station caloric densities ranged from 17.48±1.84 MJ/kg (station 10, CBL5) to 20.90±1.19 MJ/kg (station 36, UTX3), station variances ranged from 1.41 to 9.79, and standard deviations ranged from 1.19 to 3.13 (Table 2.3). Of the classes represented, bivalves had the most observations (48) across all 15 stations, while classes Amphipoda and Echinoidea (sand dollars) each only had 1 caloric observation. Echinoidea (n=1), Holothuoridea (n=2), and Ascidiacea (n=6) were the classes with the lowest mean caloric content (15.13, 16.09±0.42, and 16.11±1.48 MJ/kg, respectively), and Polychaeta (n=15), Gastropoda (n=41), and Bivalvia (n=48) were the classes with the highest mean caloric content (20.49±0.80, 20.85±0.73, and 20.98±1.31 MJ/kg, respectively). Class Holothuroidea had the lowest variance (0.18) and standard deviation (0.42) of all classes, and class Sipunculidea had the highest variance (4.22) and standard deviation (2.053) (Table 2.4). The Kruskal-Wallis rank sum test identified a significant difference ($p<0.001$) between the 11 classes of benthic and epibenthic macroinvertebrates observed in this study.

Correlations

The Spearman's Rank test found latitude ($r=0.661$; $p=0.009$) and bottom temperature ($r=-0.560$; $p=0.033$) to be significantly correlated with caloric content throughout the Chukchi Sea study area (Table 2.5). Latitude was positively correlated

with caloric content, while bottom temperature was negatively correlated. A Pearson's correlation found more significant relationships between spatial and environmental variables and caloric content (Table 2.5). Specifically, latitude (r=0.710; p=0.003), bottom water temperature (r=-0.562; p=0.029) and salinity (r=0.542; p=0.037), course sand (r=-0.591; 0.026), all sand (combined) (r=-0.547; p=0.043), silt (r=0.541; p=0.046), and sediment TON (r=0.574; p=0.032) were found to be significant. Of these variables, only caloric content, composite sand, silt, and sediment TON satisfied Anderson Darling normality tests. A correlation diagram with a matrix of pies representing r values generated by the lattice package for R (Figure 2.2) visually depicted these relationships, and also the relationships among the various spatial and environmental variables.

Linear Models and Nested Mixed Effects Model

Two linear models were created: one with the influence of taxon included, and the other with the influence of taxon screened. For the first, the Adjusted r^2 (Figure 2.3) and the Mallow's C_p analysis (Figure 2.4) suggested that the best-fit model incorporates 2 variables: 7 levels of class (Ascidiacea, Asteroidea, Bivalvia, Echinoidea, Gastropoda, Holothuroidea, and Polychaeta), and latitude. When class and latitude were incorporated into the linear model and ANOVA was applied (n=171, dof=156), latitude was a significant explanatory variable for caloric content ($p = 0.003$), though not nearly as strongly as class ($p<0.001$). A Tukey HSD test on the class variable returned 22 significant ($p<0.05$) differences out of 55 possible pairings of class levels (Table 2.6).

Gastropoda and Asteroidea had the most significant difference in caloric content ($p<0.001$).

For the second linear model, the effects of spatial and environmental variables on caloric energy were evaluated separately from taxonomic variables. Initially, a linear model was constructed to model caloric content as a function of class and the lowest taxon identified (n=171, dof=136), and taxonomic class dependency was again found to be a significant explanatory variable for caloric content ($p<0.001$). However, at finer taxonomic levels (i.e. the lowest identifiable taxon), dependency was found to be non-significant ($p=0.055$). Tukey HSD analysis revealed significant differences in 22 out of 55 possible pairings at class levels (Table 2.7). Classes Bivalvia and Ascidiacea had the most significant difference in caloric content in this model ($p<0.001$).

After the taxon dependencies were regressed out, the residuals ranged from -3.81 MJ/kg to 3.20 MJ/kg and averaged -9.77×10^{-18} MJ/kg. For the second step of this model, a linear model regressing the caloric content residuals on environmental variables was performed. The Adjusted r^2 (Figure 2.5) and the Mallow's C_p analysis (Figure 2.6) indicated that the best fit model contains 5 variables (latitude, depth, bottom water temperature, sediment TOC and TON). When these variables were incorporated into a linear model explaining caloric content, all 5 came back as significant, with 87% of variation in residual caloric content explained by the overall model. Latitude returned as the most significant explanatory variable for caloric content in the Chukchi Sea study area, followed by sediment TOC, bottom water temperature, depth, and sediment TON (see Table 2.8 for statistical values). Spatial plotting of caloric content (Figure 2.7) and

residual caloric content (Figure 2.8) indicates a northward increase in caloric content in the Chukchi Sea study area both with and without the influence of taxon.

In the mixed effects model, the environmental variables of longitude, bottom water salinity and bottom water temperature were non-significant, so they were dropped from the model. In the new model, the fixed effects of latitude and sediment chlorophyll *a* were found to be significant explanatory variables for caloric content, and the random effect of class was found to be significant, as the high posterior density (HPD) 95% confidence interval (0.742-1.432) did not include the origin. The random effect of lowest taxon nested within class was found to be non-significant at the α=0.05 level.

Cluster Analysis

The partitioning approach to cluster analysis produced two different clusterings of the 14 stations with available environmental data. The K-means curve of sum of squares versus number of clusters did not possess a sharply-defined inflection (Figure 2.9), but changed slopes at approximately 6 cluster groups. Using K-means, 6 clusters containing 1 to 6 stations per cluster were mapped with 86% variance among the clusters, and 14% variance within clusters (Figure 2.10). Clusters produced by K-means partitioning can be described as follows: Cluster group K1 contained station 30 (UTX11), K2 contained station 4 (RDM), K3 contained stations 8 (107), 27 (HSH1), and 32 (UTX5), K4 contained station 10 (CBL5), K5 contained 16 (CBL4), 18 (UTX16), 24 (CBL15), 34 (1030), 36 (UTX3), and 38, (CBL8), and K6 contained stations 6 (CBL1) and 20 (1014) (Table 2.9).

Cluster groups K1, K3 and K5 located to the north of the study area, had the highest caloric densities, while cluster groups K2, K4 and K6, located further to the south and along the Alaskan coast, had the lowest caloric densities (Figure 2.10). The higher caloric density northern clusters (K1, K3, and K5) had lower bottom water temperatures and higher salinities than the averages for all 14 stations. The lower caloric density southern clusters along the Alaskan coast (K2, K4, and K6) were uniformly shallow, warm with respect to bottom seawater temperatures, and less saline) than the averages for all 14 stations. Notably, cluster group K5, mostly located at the northwestern portion of the study area, had the highest caloric density, the greatest depth, the highest sediment chlorophyll *a*, the highest percentage of silt, the highest sediment TOC and the highest sediment TON of all cluster groups (Table 2.9). Conversely, cluster group K4, located in the central portion of the study area near the Alaskan coast, had the lowest caloric density, the lowest percentage of silt, the lowest sediment TOC, and the lowest sediment TON of all cluster groups.

Under PAM clustering, a maximum ASW was achieved with 4 clusters (Figure 2.11), with 37% dissimilarity (by ASW). The PAM cluster algorithm produced 4 clusters, each containing 1 to 6 stations (Figure 2.12). Under this approach to partitioned clustering, the groups produced were as follows: cluster group P1 contained stations 4 (RDM), 6 (CBL1), and 20 (1014), group P2 contained stations 8 (107), 10 (CBL5), 27 (HSH1), and 32 (UTX5), group P3 contained stations 16 (CBL4), 18 (UTX16), 24 (CBL15), 34 (1030), 36 (UTX3), and 38 (CBL8), and cluster group P4 contained only station 30 (UTX11) (Table 2.10).

Cluster groups P2 and P3, located mostly in the northwestern section of the study area, had the highest caloric densities, while cluster group P1, located mostly to the south and near the Alaskan coast, had the only average caloric density that was below the mean of all 14 clustered stations (Table 2.10). The higher caloric density northwestern clusters (P2 and P3) had uniformly lower temperatures, and the low caloric density cluster P1 had the only bottom water temperature above the mean. In addition, cluster group P1 was the only group with a salinity below the mean of all stations, and had the highest C:N ratio (Table 2.10.).

Discussion

Comparisons to Literature Values

Significant differences in caloric content among classes were found both by Tukey HSD testing and Kruskal Wallis one way ANOVA analysis. This is reflected in the finding that the strongest explanatory variable for energy content is taxon (class, specifically). The wide variety of taxa identified and insufficient observation numbers to evaluate them properly likely contributed to the finding that "lowest taxon identified" is not a significant explanatory variable for caloric content.

In the Canadian Arctic, a caloric survey including 10 of the same classes included in this investigation indicated similar caloric values to those measured here. In that study, tunicates, sea stars, and sand dollars measured at low caloric densities (1.62, 2.65, and 2.97 kcal/g), and bivalves and gastropods measured at high caloric densities (4.18

and 4.49 kcal/g) (Wacasey and Atkinson 1987). In this current study, sand dollars had the lowest caloric density of any class (3.62 kcal/g), followed by sea cucumbers (3.85 kcal/g) and tunicates (3.85 kcal/g). Amphipods in the Canadian Arctic have comparatively higher caloric densities (4.05 kcal/g) (Szaniawska and Wolowicz 1986), and that fact was also evident in this current study, as amphipods measured as the 4th highest mean caloric density of the 11 classes surveyed (4.66 kcal/g).

In Stoker's (1978) survey of the Pacific Arctic Region, sipunculids were estimated at low caloric densities (3.01 kcal/g), together with tunicates, polychaetes and decapods (3.57, 3.60, and 3.91 kcal/g, respectively). In his investigation, amphipods, gastropods and bivalves had the highest caloric densities (4.66, 4.90, and 4.98 kcal/g). The results of the current study are similar, with low caloric densities measured for sipunculids (4.40 kcal/g) and tunicates (3.85 kcal/g), and high caloric densities for amphipods, gastropods and bivalves (4.66, 4.98, and 5.014 kcal/g). Unlike Stoker's finding that polychaete worms were relatively low in caloric content, polychaete worms in this investigation measured at relatively high caloric densities (4.90 kcal/g). It must be noted in this comparison that Stoker's caloric values were based upon samples preserved in formalin, whereas the animals in this study were preserved by freezing. Significant differences in caloric content may exist between samples preserved under differing preservation methods (Hondolero et al. 2011). This question is explored further in chapter 3.

In addition to a large caloric survey on formalin-preserved benthic taxa, Stoker (1978) also conducted a smaller caloric survey on frozen specimens from 9 bivalve taxa and 6 other miscellaneous taxa, including two polychaete worms (F. Maldanidae and F.

Nephtyidae). The average caloric value for frozen bivalves was 4.42 kcal/g (Stoker 1978), while the average caloric value for frozen bivalves surveyed in this current study was much higher (5.014 kcal/g). The average caloric value between the two polychaete taxa in Stoker's survey (3.64 kcal/g) (Stoker 1978) is also less than the finding of this current survey (4.90 kcal/g). It is likely that discrepancies between these measurements are either due to differences in instrumentation, geography (Gallagher et al. 1998), species found, or sampling season (Mann 1978, Okumus and Stirling 1998). Stoker's field sampling was conducted over a 4-year period in both summer and winter. Because the lipid content of polychaete worms is known to depend largely upon diet (Luis et al. 1995), strong seasonal changes in Chukchi Sea annual primary production would likely result in seasonal differences in polychaete lipid content, and therefore caloric content. Different polychaete species may also feed at different trophic levels, which may also lead to differences in caloric content.

Relationships between Caloric Content and Physical, Biological and Spatial Variables

In this investigation, differences in grain size (% sand and silt) had significant relationships to caloric content by Pearson's correlation (Table 2.5). These results are likely related to habitat preference by key prey species with high lipid content. For example, bivalves are known to contain higher lipid levels than other macroinvertebrate fauna (Parrish et al. 1996). In Svalbard, the vast majority of bivalves exhibit strong preferences for soft sediment, and only a small percentage (including *Hiatella* and *Mya*) were found on both hard and soft substrata (Wlodarska-Kowalczuk 2007). These and

other analyses concluded that deposit-feeding bivalves prefer softer sediments because smaller grain sizes result in fluidized sediments that facilitate access to particulate organic carbon (Weston 1988). Sediment type has also been identified as a key variable in cluster analysis for both infauna (Grebmeier et al. 1989, Feder et al. 1994, 2007) in the southern and northern Chukchi Sea, respectively, and epifaunal taxa in the southern Chukchi Sea (Feder et al. 1994, 2005) and northern Bering Sea (Konar in final report COMIDA CAB, cite project website), though the largest group by biomass of epifauna were the echinoderms (Bluhm et al. 2009).

Of the biological variables, sediment TOC, TON, and chlorophyll *a* were found to have significant relationships to caloric content. Both sediment TOC and TON positively correlated to caloric content, though neither was found to be significant with alpha set at 0.05. Despite non-significant findings in correlation analysis, the best fit model for residual energy (with the effects of taxon regressed out) included both sediment TOC and TON as significant explanatory variables for caloric content ($p=0.002$, $p=0.005$), suggesting that the importance of sediment TOC and TON may have been obscured in correlation by the massive dependency upon taxon. Further supporting the importance of sediment TOC and TON, under K-means cluster analysis, the cluster with the highest caloric density of all 6 clusters also had the highest sediment TOC and TON content of all 6 clusters, and the cluster with the lowest caloric density of all 6 clusters also had the lowest TOC and TON of all 6 clusters (Table 2.9).

Sediment TOC and TON can be interpreted as representative of food availability in the Chukchi Sea study area, thus supporting their importance to driving prey caloric content. When food availability is high, the lipid content of invertebrates is known to

increase (Luis et al. 1995), and organisms with higher lipid content measure at higher caloric densities than animals with low lipid content (Falk-Petersen et al. 1990; Weslawski et al. 2010). Sediment chlorophyll *a* was also identified as a significant explanatory variable for caloric content ($p=0.041$) in the mixed effects model which likely reflects the importance of food availability to macroinvertebrates when evaluating caloric content as it does with benthic standing stock (Grebmeier et al. 2006a).

Bottom temperature also returned as a significant explanatory variable for caloric content likely due to its strong relationship to latitude in the Chukchi Sea study area (Figure 2.2). Latitude was consistently the strongest non-taxonomic predictor of caloric content in this investigation, which coincides with the observed higher benthic infaunal carbon biomass in the region (Grebmeier et al. 2006a). Higher concentrations of lipid rich bivalves have also been observed in the northern part of the Chukchi (Grebmeier 2012). The finding that latitude is the strongest non-taxonomic dependency for caloric content is also likely related to the spatial distribution of water masses throughout the Chukchi Sea study area. To the south of the COMIDA CAB study area and along the Alaskan coast, the low nutrient ACW water mass flows northward from the Bering Strait, and in the northwest portion of the COMIDA CAB study area, the higher nutrient BSAW (Bering Sea water in the Chukchi Sea; Weingartner et al. 2005) transits first west, then north, and then heads northeast in the northern sector of the Chukchi Sea (Coachman 1987; McRoy 1993; Weingartner et al. 2005). The BSAW water mass is known to support a higher water column production and subsequent export of carbon to the benthos (Grebmeier et al. 2006a), resulting in higher benthic productivity in northern region than in the ACW water mass (Feder et al. 1994a, b; Grebmeier 2012; Grebmeier et al 2006a.

Cluster analysis also highlights the importance of water mass to caloric content in the Chukchi Sea study area. Nearly all of the stations in the two K-means cluster groups with the highest caloric densities (K3 and K5, Table 2.9, Figure 2.10) were located within the BSAW water mass, while the two K-means cluster groups with the lowest caloric densities (K2 and K4, Table 2.9, Figure 2.10) were both located to the south near the Alaskan coast. Under PAM cluster analysis, the stations in the 2 cluster groups with the highest caloric densities (P2, P3, Table 2.10, Figure 2.12) were almost all (with the exception of 1 out of 10 stations) located within the BSAW water mass, while the 3 stations in the cluster group with the lowest caloric density (P1, Table 2.10, Figure 2.12) were mostly located south and along the Alaskan coast.

Relevance to Pacific Walrus and Other Higher Trophic Level Organisms

Walrus consume wide variety of benthic organisms, but prefer softer-bodied bivalves, gastropods and polychaete worms (Sheffield and Grebmeier 2009), which are also high in lipid content (Parrish et al. 1996). Higher lipid content organisms are associated with higher caloric densities (Stoker 1978; Percy and Fife 1980; Weslawski et al. 2010).

The latitudinal associations with caloric content identified in this survey may have important implications for higher trophic level predators, particularly Pacific walrus. Walruses rely upon seasonal ice floes in the marginal ice zone for transport to preferred feeding grounds and for resting platforms during hunting (Fay 1982). Hauling out on Alaskan and Russian shores as a response to decreasing amounts of sea ice (Jay et al.

2011) separates walrus geographically from the highest quality benthic prey, based upon caloric density, within their traditional feeding grounds. This study indicates that caloric densities of benthic prey are highest offshore and to the northwest in the COMIDA CAB study area. This finding may also explain satellite telemetry data showing walruses making energetically costly efforts to reach these feeding grounds from land (Jay et al. in prep.).

These caloric data will therefore be helpful for modeling foraging energetics for walrus and other higher trophic level predators such as bottom feeding fish, whales, seals, and diving birds that are known to consume infaunal and epifaunal macroinvertebrates (Lowry et al. 1980; Hazard and Lowry 1984; Highsmith and Coyle 1992; Lovvorn et al. 2003; Cui et al. 2009; Iken et al. 2010). Understanding foraging energetics for Pacific walrus in particular will enhance our understanding of cost and benefit tradeoffs associated with walrus traveling from haul-out sites to preferred feeding grounds.

Conclusions

Linear models and mixed effects modeling confirmed the alternate, first hypothesis of this investigation that taxon is the most significant explanatory variable for caloric content. This is likely due to the higher lipid levels that are found in softer bodied macroinvertebrate organisms. Significant differences were found to exist between the classes by Kruskal Wallis testing, and Tukey HSD tests on ANOVA models confirmed that significant differences exist between 22 specific class level pairings. The second alternate hypothesis, that higher sediment TOC and TON result in higher caloric

densities, was confirmed by linear modeling on residual caloric content, once taxonomic effects were regressed out. This was also corroborated by the K-means cluster analysis. Caloric content was found in multiple statistical analyses to increase with increasing latitude in the COMIDA CAB area, confirming the third alternate hypothesis of this study. As indicated by cluster analysis, the connection between caloric content and latitude in this study area is likely related to water mass type (Anadyr Water) that is characterized by higher water column nutrients and primary productivity, along with colder bottom water temperatures, all conducive to increasing carbon export to maintain higher benthic biomass and more caloric-rich benthic taxa. This confirms the fourth alternative hypothesis. The connection that this finding has to altered Pacific walrus foraging patterns may prove valuable in structuring foraging energetics assessments, which may be a powerful tool to evaluate the outlook for Pacific walrus in the face of changing environmental conditions.

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Tables

Table 2.1: Sampling stations and associated environmental parameters for the 15 Chukchi Sea Offshore Monitoring in Drilling Area (COMIDA) Chemical and Benthos (CAB) stations chosen for caloric analysis.

Station Number	Station Name	Zone	Quadrant	Collection Date (mm/dd/yyyy)	Latitude (°N)	Longitude (°W)	Depth (m)	Bottom Temperature (°C)	Bottom Salinity (psu)
4	RDM	Nearshore	Southwest	7/27/2010	67.562	-164.178	18	7.38	30.58
6	CBL1	Nearshore	Southwest	7/28/2010	69.04	-166.594	35	6.08	31.25
8	107	Nearshore	Southwest	7/29/2010	70.086	-166.455	47	0.14	31.94
10	CBL5	Nearshore	Southeast	7/29/2010	70.023	-163.761	27	0.86	32.15
16	CBL4	Midshore	Southwest	7/31/2010	70.831	-167.787	55	-1.47	32.46
18	UTX16	Midshore	Northwest	8/1/2010	71.249	-165.448	43	-1.38	32.46
20	1014	Nearshore	Southeast	8/1/2010	70.84	-163.291	45	0.22	32.16
21	CBL16	Nearshore	Northeast	8/3/2010	71.414	-157.491	126	-0.87	32.77
24	CBL15	Midshore	Northeast	8/4/2010	71.727	-160.718	45	-1.63	32.86
27	HSH1	Midshore	Northeast	8/5/2010	72.101	-162.975	36	-1.63	32.67
30	UTX11	Midshore	Northeast	8/5/2010	71.453	-162.611	44	-1.67	32.84
32	UTX5	Midshore	Northwest	8/6/2010	71.702	-164.515	38	-1.54	32.66
34	1030	Offshore	Northwest	8/6/2010	72.103	-165.456	45	-1.36	32.49
36	UTX3	Offshore	Northwest	8/7/2010	71.93	-167.389	48	-1.76	32.84
38	CBL8	Offshore	Northwest	8/7/2010	71.485	-167.782	48	-1.69	32.7

Table 2.2: Spatial and environmental data for all stations surveyed for caloric content during the July-August 2010 COMIDA CAB cruise.

Station Number	Station Name	Average Caloric Content (MJ/kg)	Latitude (°N)	Longitude (°W)	Depth (m)	Bottom Temperature (°C)	Bottom Salinity (psu)	Sediment Chlorophyll A (mg/m²)	Sedimet Grain Size <0 Φ (%)	Sediment Grain Size 1 Φ (%)
4	RDM	18.309	67.562	-164.178	18	7.38	30.58	31.88	0	0.19
6	CBL1	18.767	69.04	-166.594	35	6.08	31.25	9.63	0.14	0.29
8	107	19.73	70.086	-166.455	47	0.14	31.94	6.24	0.58	0.53
10	CBL5	17.48	70.023	-163.761	27	0.86	32.15	9.31	0.09	0.6
16	CBL4	19.471	70.831	-167.787	55	-1.47	32.46	30.45	0.05	0.05
18	UTX16	20.339	71.249	-165.448	43	-1.38	32.46	44.68	0.5	0.15
20	1014	19.222	70.84	-163.291	45	0.22	32.16	16.95	0.14	0.24
21	CBL16	19.441	71.414	-157.491	126	-0.87	32.77	-	-	-
24	CBL15	20.279	71.727	-160.718	45	-1.63	32.86	17.01	0	0.05
27	HSH1	20.183	72.101	-162.975	36	-1.63	32.67	6.92	0.78	1.01
30	UTX11	19.581	71.453	-162.611	44	-1.67	32.84	9.19	3.19	2.39
32	UTX5	20.073	71.702	-164.515	38	-1.54	32.66	7.56	1.34	0.23
34	1030	19.451	72.103	-165.456	45	-1.36	32.49	59.87	0.65	0.1
36	UTX3	20.896	71.93	-167.389	48	-1.76	32.84	42.24	0	0.05
38	CBL8	20.116	71.485	-167.782	48	-1.69	32.7	41.59	0	0.05

Station Number	Station Name	Sediment Grain Size 2 Φ (%)	Sediment Grain Size 3 Φ (%)	Sediment Grain Size 4 Φ (%)	Sand (Grain Size 1-4 Φ) (%)	Silt Grain Size >5 Φ (%)	Sediment Modal Size	Sediment TOC (%)	Sediment TON (%)	Sediment C:N Ratio
4	RDM	0.99	3.67	52.56	57.4	42.6	4	0.41	0.06	6.83
6	CBL1	0.57	2.71	31.4	34.96	64.89	5	1.1	0.12	9.17
8	107	6.15	36.43	20.83	63.95	35.47	3	0.46	0.06	7.67
10	CBL5	22.2	57	7.12	86.92	12.98	3	0.13	0.02	6.5
16	CBL4	0.14	2.32	22.66	25.16	74.79	5	0.97	0.13	7.46
18	UTX16	0.75	18.17	16.53	35.59	63.91	5	0.88	0.13	6.77
20	1014	3.47	11.82	37.29	52.82	47.03	5	0.58	0.07	8.29

21	CBL16	-	-	-	-	-	-	-	-	-
24	CBL15	0.05	0.84	4.5	5.45	94.55	5	1.35	0.21	6.43
27	HSH1	7.82	41.63	12.31	62.76	36.46	3	0.41	0.06	6.83
30	UTX11	6.89	12.61	10.07	31.96	64.85	5	0.88	0.12	7.33
32	UTX5	1.52	53.25	16.44	71.44	27.22	3	0.36	0.05	7.2
34	1030	0.1	1.06	8.06	9.32	90.03	5	1.48	0.19	7.79
36	UTX3	0.05	0.72	5.27	6.09	93.91	5	1.47	0.2	7.35
38	CBL8	0.1	1.01	8.52	9.69	90.31	5	1.24	0.18	6.89

Table 2.3: Mean caloric content (MJ/kg) and associated statistics for stations surveyed during the July-August 2010 COMIDA CAB cruise.

Station Number	Station Name	Mean Caloric Content (MJ/kg)	Minimum Caloric Observation (MJ/kg)	Maximum Caloric Observation (MJ/kg)	Variance	Standard Deviation	Number of Caloric Observations	Number of Classes Found at Station
4	RDM	18.309	14.946	21.288	6.746	2.597	6	4
6	CBL1	18.767	15.072	22.383	9.793	3.129	7	5
8	107	19.730	15.174	23.226	7.954	2.820	8	6
10	CBL5	17.480	15.133	20.769	3.376	1.837	6	5
16	CBL4	19.471	16.063	21.765	3.471	1.863	14	6
18	UTX16	20.339	15.152	22.063	5.430	2.330	11	4
20	1014	19.222	16.040	22.251	5.372	2.318	13	8
21	CBL16	19.441	17.485	21.459	1.839	1.356	6	4
24	CBL15	20.279	17.069	21.878	2.649	1.627	16	6
27	HSH1	20.183	13.698	23.244	7.748	2.783	10	5
30	UTX11	19.581	15.792	21.324	2.290	1.513	18	7
32	UTX5	20.073	17.413	22.560	2.274	1.508	12	6
34	1030	19.451	14.240	22.293	3.822	1.955	18	6
36	UTX3	20.896	18.414	22.673	1.409	1.187	13	5
38	CBL8	20.116	15.824	23.494	4.584	2.141	13	6

Table 2.4: Caloric observations for infaunal and epifaunal animals collected during the July-August 2010 COMIDA CAB cruise.

Class	Number of Caloric Observations	Mean Caloric Content (MJ/kg)	Minimum Caloric Observation (MJ/kg)	Maximum Caloric Observation (MJ/kg)	Variance	Standard Deviation
Amphipoda	1	19.476	-	-	-	-
Anthozoa	5	18.521	16.679	20.063	1.556	1.247
Ascidiacea	6	16.113	13.698	17.485	2.189	1.479
Asteroidea	11	16.725	14.240	19.928	2.673	1.635
Bivalvia	48	20.978	17.595	23.494	1.721	1.312
Echinoidea	1	15.133	-	-	-	-
Gastropoda	41	20.848	18.928	22.383	0.539	0.734
Holothuroidea	2	16.089	15.792	16.387	0.177	0.421
Malacostraca	35	18.501	14.946	23.244	3.591	1.895
Polychaeta	15	20.493	18.997	21.862	0.643	0.802
Sipunculidea	6	18.419	16.251	21.469	4.217	2.053

Table 2.5: Correlation table of caloric infaunal values with environmental variables. Significant values ($p \leq 0.05$) are bolded. Animals were collected during the July-August 2010 COMIDA CAB cruise.

Variable	Spearman r	Spearman p-value	Pearson r	Pearson p-value
Latitude (°N)	**0.661**	**0.009**	**0.710**	**0.003**
Longitude (°W)	-0.136	0.630	-0.122	0.665
Depth (m)	0.341	0.214	0.221	0.429
Bottom Temperature (°C)	**-0.560**	**0.033**	**-0.562**	**0.029**
Bottom Salinity (psu)	0.424	0.117	**0.542**	**0.037**
Sediment Chlorophyll A (mg/m²)	0.156	0.594	0.255	0.379
Grain Size <0 Φ (%)	-0.022	0.945	0.126	0.667
Grain Size 1 Φ (%)	-0.384	0.176	-0.095	0.746
Grain Size 2 Φ (%)	-0.401	0.157	**-0.591**	**0.026**
Grain Size 3 Φ (%)	-0.270	0.349	-0.225	0.439
Grain Size 4 Φ (%)	-0.459	0.101	-0.455	0.102
Sand (Grain Size 1-4 Φ) (%)	-0.402	0.155	**-0.547**	**0.043**
Grain Size >5 Φ (%)	0.407	0.150	**0.541**	**0.046**
Sediment Modal Size	0.182	0.533	0.261	0.368
Sediment TOC (%)	0.357	0.211	0.512	0.061
Sediment TON (%	0.509	0.066	**0.574**	**0.032**
Sediment C:N Ratio	-0.304	0.291	-0.128	0.663

Table 2.6: Class level comparison of benthic macroinvertebates with environmental parameters usng a Tukey's Honest Significant Differences (HSD) test organized by ascending p-value. Animals were collected during the July-August 2010 COMIDA CAB cruise.

Class Level Pairing	**p-value**
Bivalvia-Ascidiacea	<0.001
Bivalvia-Asteroidea	<0.001
Gastropoda-Ascidiacea	<0.001
Gastropoda-Asteroidea	<0.001
Holothuroidea-Bivalvia	<0.001
Holothuroidea-Gastropoda	<0.001
Malacostraca-Bivalvia	<0.001
Malacostraca-Gastropoda	<0.001
Polychaeta-Ascidiacea	<0.001
Polychaeta-Asteroidea	<0.001
Polychaeta-Malacostraca	<0.001
Polychaeta-Holothuroidea	0.001
Bivalvia-Anthozoa	0.005
Gastropoda-Anthozoa	0.006
Malacostraca-Ascidiacea	0.006
Echinoidea-Bivalvia	0.007
Gastropoda-Echinoidea	0.008
Malacostraca-Asteroidea	0.008
Sipunculidea-Bivalvia	0.02
Polychaeta-Echinoidea	0.023
Sipunculidea-Gastropoda	0.023
Sipunculidea-Ascidiacea	0.033

Table 2.7: Class level benthic macroinvertebrate pairings with significant differences produced by Tukey's Honest Significant Differences (HSD) test from the regressed out portion of a linear model explaining caloric content, organized by ascending p-value. Animals were collected during the July-August 2010 COMIDA CAB cruise.

Class Level Pairing	**p-value**
Bivalvia-Ascidiacea	<0.001
Bivalvia-Asteroidea	<0.001
Gastropoda-Ascidiacea	<0.001
Gastropoda-Asteroidea	<0.001
Holothuroidea-Bivalvia	<0.001
Holothuroidea-Gastropoda	<0.001
Malacostraca-Bivalvia	<0.001
Malacostraca-Gastropoda	<0.001
Polychaeta-Ascidiacea	<0.001
Polychaeta-Asteroidea	<0.001
Polychaeta-Malacostraca	<0.001
Sipunculidea-Bivalvia	<0.001
Polychaeta-Holothuroidea	0.001
Echinoidea-Bivalvia	0.001
Gastropoda-Echinoidea	0.002
Sipunculidea-Gastropoda	0.002
Malacostraca-Ascidiacea	0.003
Bivalvia-Anthozoa	0.004
Polychaeta-Echinoidea	0.006
Malacostraca-Asteroidea	0.006
Gastropoda-Anthozoa	0.012
Sipunculidea-Polychaeta	0.050

Table 2.8: ANOVA output for a model explaining residual caloric content (without taxonomic influence). Animals were collected during the July-August 2010 COMIDA CAB cruise.

Variable	**Coefficient Estimate**	**Standard Error**	**t-value**	**p-value**
Intercept	-62.446	12.147	-5.141	<0.001
Latitude	0.857	0.169	5.077	<0.001
Sediment TOC	-5.273	1.195	-4.412	0.002
Bottom Temperature	0.395	0.096	4.099	0.003
Depth	0.065	0.017	3.954	0.004
Sediment TON	29.368	7.630	3.849	0.005
Residual Standard Error	0.252			
Degrees of Freedom	8.000			
Multiple r^2	0.870			
Adjusted r^2	0.788			
F-statistic	10.670			
p-value	0.002			
n	15			

Table 2.9: Summary of the 6 cluster groups produced by K-means cluster analysis, with 86% variance between clusters. All variables normalized in 0 mean and 1 standard deviation. Animals were collected during the July-August 2010 COMIDA CAB cruise.

Cluster Identifier	Stations Numbers	Average Caloric Content	Depth	Bottom water Temperature	Bottom water Salinity	Sediment Chlorophyll A	Sediment Grain Size <0 Φ	Sediment Grain Size 1 Φ	Sediment Grain Size 2 Φ
K1	30	0.019	0.314	-0.648	0.936	-0.826	3.082	3.129	0.542
K2	4	-1.393	-2.408	2.443	-2.595	0.455	-0.618	-0.372	-0.438
K3	8, 27, 32	0.479	-0.070	-0.422	0.285	-0.955	0.426	0.265	0.255
K4	10	-2.314	-1.466	0.216	-0.142	-0.819	-0.514	0.281	3.085
K5	16, 18, 24, 34, 36, 38	0.586	0.663	-0.457	0.436	0.874	-0.386	-0.555	-0.570
K6	6, 20	-0.632	-0.105	0.998	-0.837	-0.595	-0.456	-0.252	-0.267

Cluster Identifier	Station Numbers	Sediment Grain Size 3 Φ	Sediment Grain Size 4 Φ	Sand (Grain Size 1-4 Φ)	Sediment Grain Size >5 Φ	Sediment Modal Size	Sediment TOC	Sediment TON	Sediment C:N Ratio
K1	30	-0.230	-0.577	-0.284	0.183	0.692	0.095	0.091	0.011
K2	4	-0.662	2.471	0.669	-0.646	-0.385	-0.950	-0.867	-0.663
K3	8, 27, 32	1.274	-0.114	0.992	-1.002	-1.461	-0.950	-0.920	-0.120
K4	10	1.913	-0.788	1.774	-1.750	-1.461	-1.572	-1.506	-1.107
K5	16, 18, 24, 34, 36, 38	-0.645	-0.516	-0.910	0.919	0.692	0.877	0.943	-0.279
K6	6, 20	-0.488	1.164	0.163	-0.148	0.692	0.006	-0.308	1.895

Table 2.10: Summary of the four cluster groups produced by Partitioning Around Medioids (PAM) cluster analysis, with 37% dissimilarity by average silhouette width (ASW). All variables normalized in 0 mean and 1 standard deviation. Animals were collected during the July-August 2010 COMIDA CAB cruise.

Cluster Identifier	Stations Numbers	Average Caloric Content	Depth	Bottom Temperature	Bottom Salinity	Sediment Chlorophyll A	Grain Size <0 Φ	Grain Size 1 Φ	Grain Size 2 Φ
P1	4, 6, 20	-0.884	-0.628	1.999	-1.548	-0.801	-0.456	-0.213	-0.508
P2	8, 10, 27, 32	0.686	-0.523	-0.634	0.671	-0.954	0.287	0.933	0.696
P3	16, 18, 24, 34, 36, 38	0.613	0.733	-0.654	0.718	1.003	-0.618	-0.594	-0.586
P4	30	0.019	0.314	-0.648	0.936	-0.826	3.082	3.129	0.542

Cluster Identifier	Station Numbers	Grain Size 3 Φ	Grain Size 4 Φ	Sand (Grain Size 1-4 Φ)	Grain Size >5 Φ	Sediment Modal Size	Sediment TOC	Sediment TON	Sediment C:N Ratio
P1	4, 6, 20	-0.708	0.953	-0.171	0.185	0.692	0.584	0.091	2.488
P2	8, 10, 27, 32	1.171	-0.416	0.869	-0.875	-1.461	-0.950	-0.867	-0.663
P3	16, 18, 24, 34, 36, 38	-0.790	-0.688	-1.117	1.132	0.692	0.896	1.050	-0.582
P4	30	-0.230	-0.577	-0.284	0.183	0.692	0.095	0.091	0.011

Figures

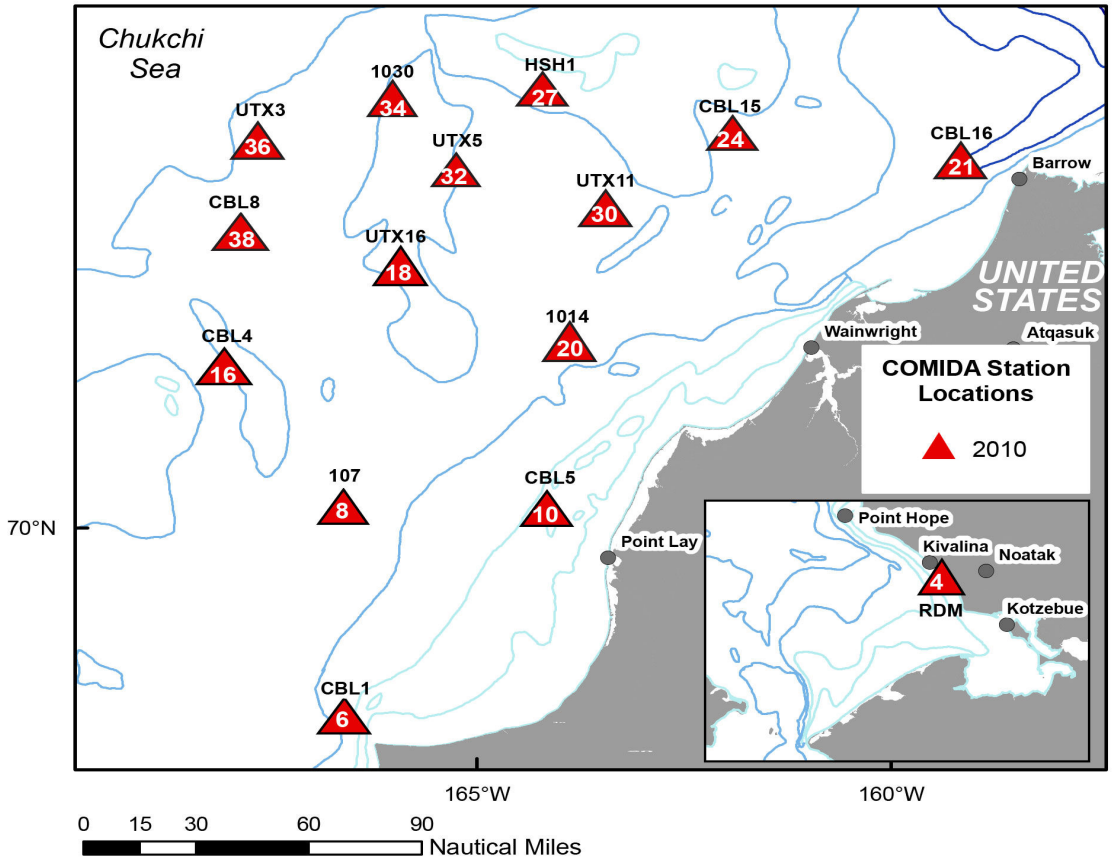

Figure 2.1: Map of stations analyzed for caloric content from the Chukchi Sea Offshore Monitoring in Drilling Area (COMIDA) Chemical and Benthos (CAB) project study area.

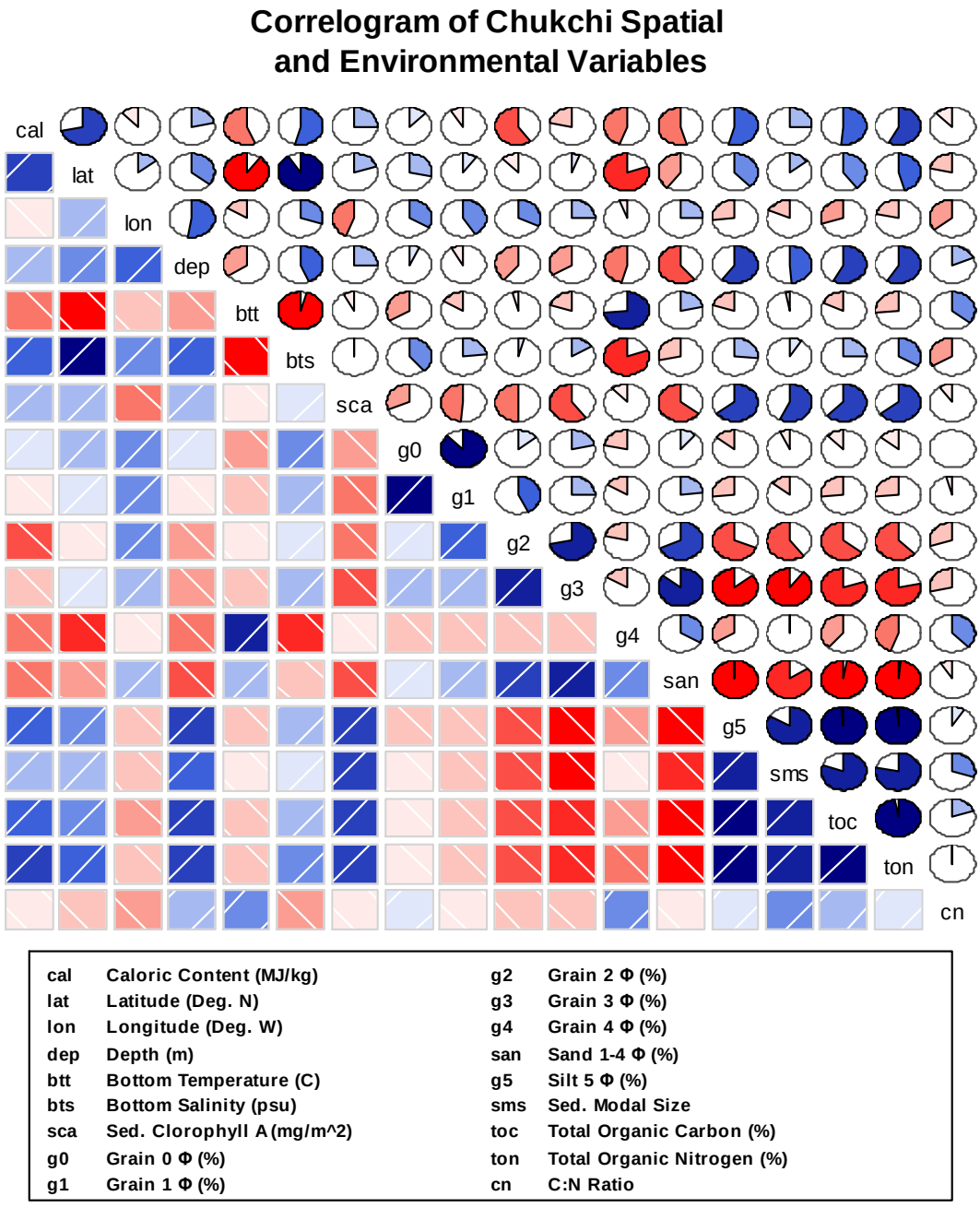

Figure 2.2: Correlation diagram for faunal caloric content against 18 spatial and environmental variables, with pies representing r values (blue is positive, red is negative). Squares also represent r values, with positive relationships sloping to the top right and negative relationships sloping to the top left. TOC=total organic carbon and TON=total organic nitrogen in surface sediments. Animals were collected during the July-August 2010 COMIDA CAB cruise.

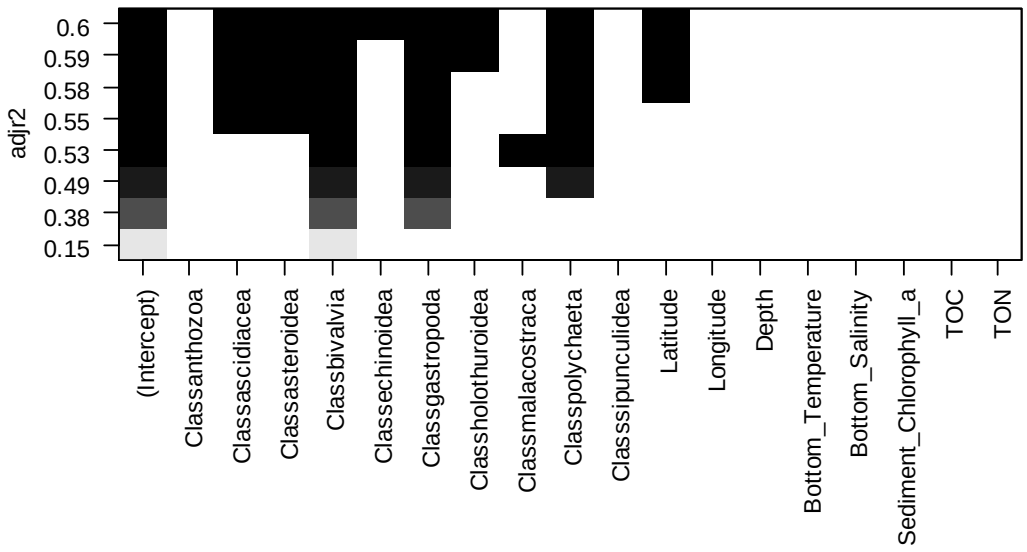

Figure 2.3: Adjusted r^2 best fit model for a linear model with taxon related dependencies. Key: Sediment total organic carbon (TOC) and nitrogen (TON). Animals were collected during the July-August 2010 COMIDA CAB cruise.

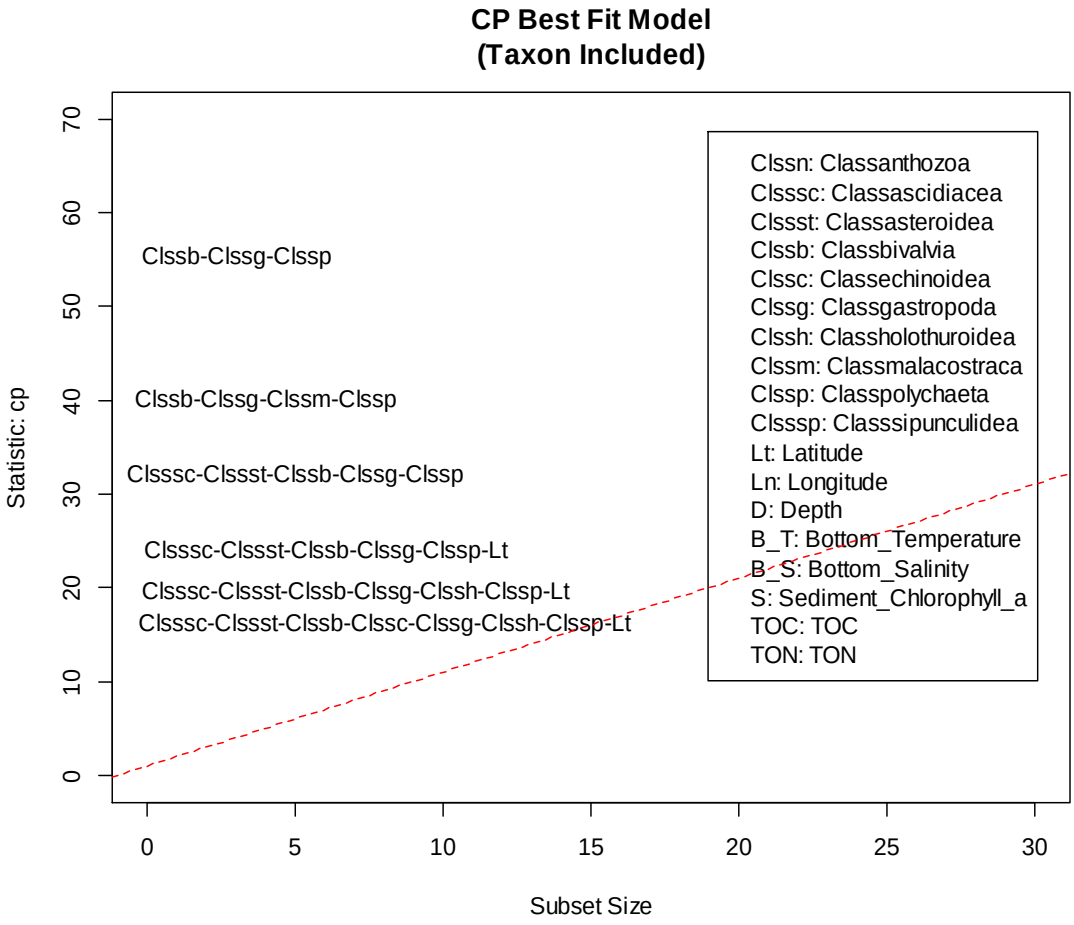

Figure 2.4: C_p best fit model for a linear model with taxon related dependencies. Key: Sediment total organic carbon (TOC) and nitrogen (TON). Animals were collected during the July-August 2010 COMIDA CAB cruise.

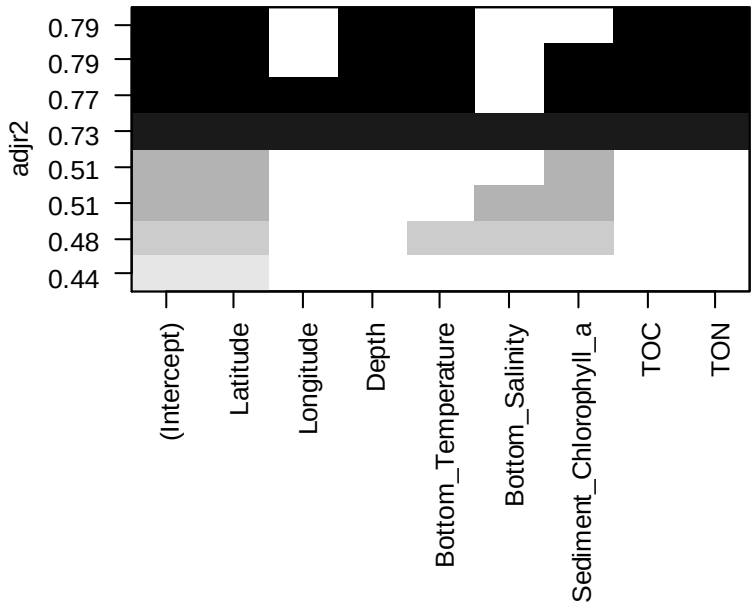

Figure 2.5: Adjusted r^2 best fit model for a linear model with taxon related dependencies regressed out. Key: Sediment total organic carbon (TOC) and nitrogen (TON). Animals were collected during the July-August 2010 COMIDA CAB cruise.

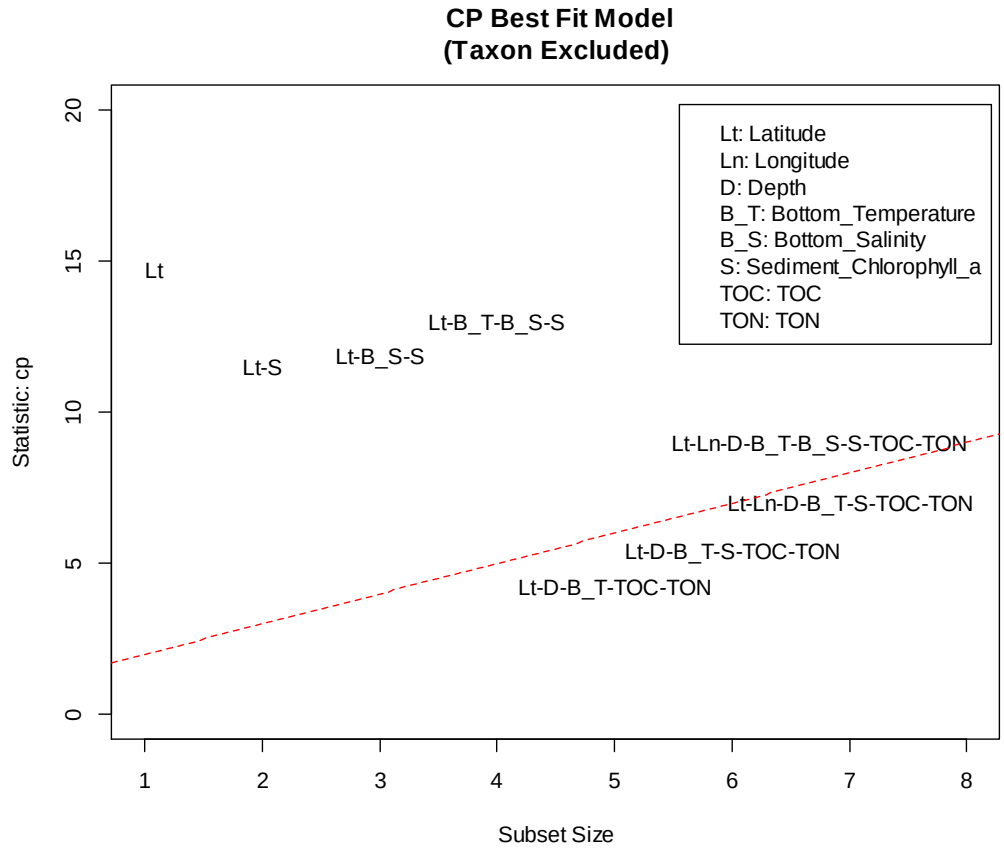

Figure 2.6: C_p best fit model for a linear model with taxon related dependencies. Key: Sediment total organic carbon (TOC) and nitrogen (TON). Animals were collected during the July-August 2010 COMIDA CAB cruise.

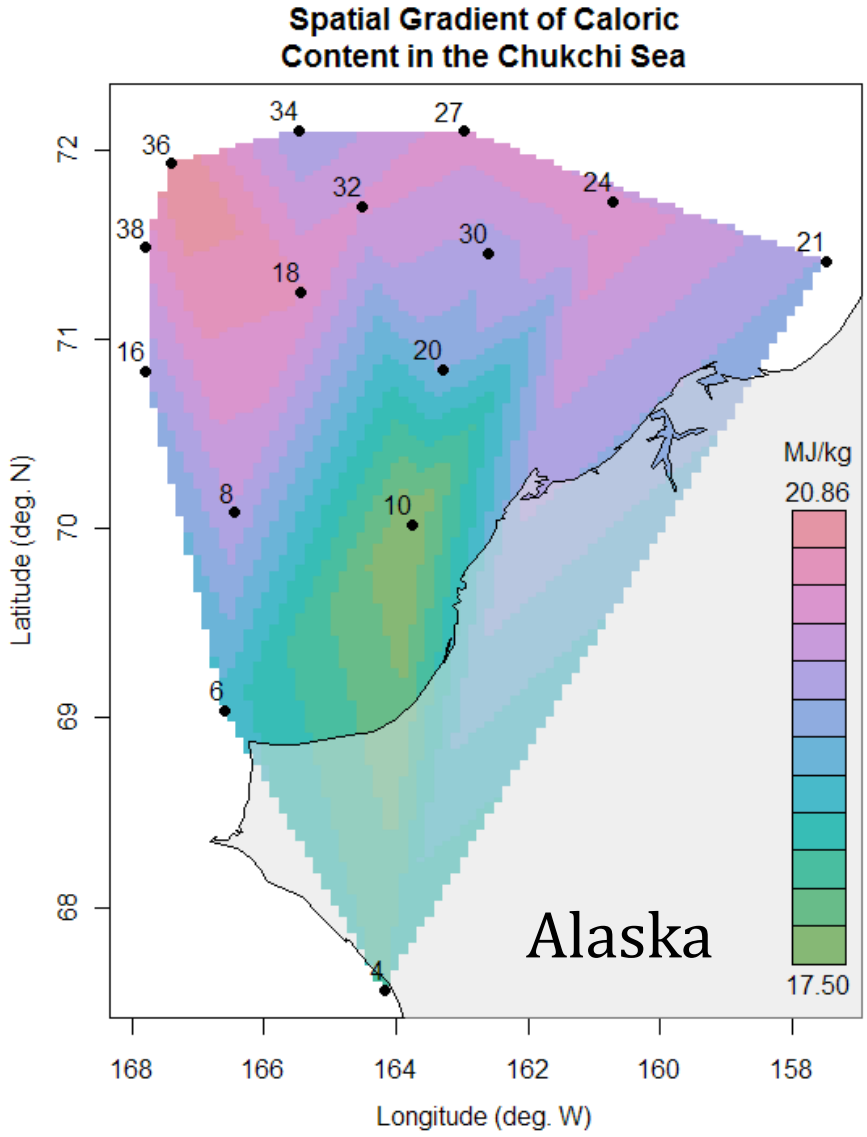

Figure 2.7: Spatially interpolated plot of caloric content (including the influence of taxonomic variables) for animals collected during the July-August 2010 COMIDA CAB cruise. Black dots are stations surveyed, with station number.

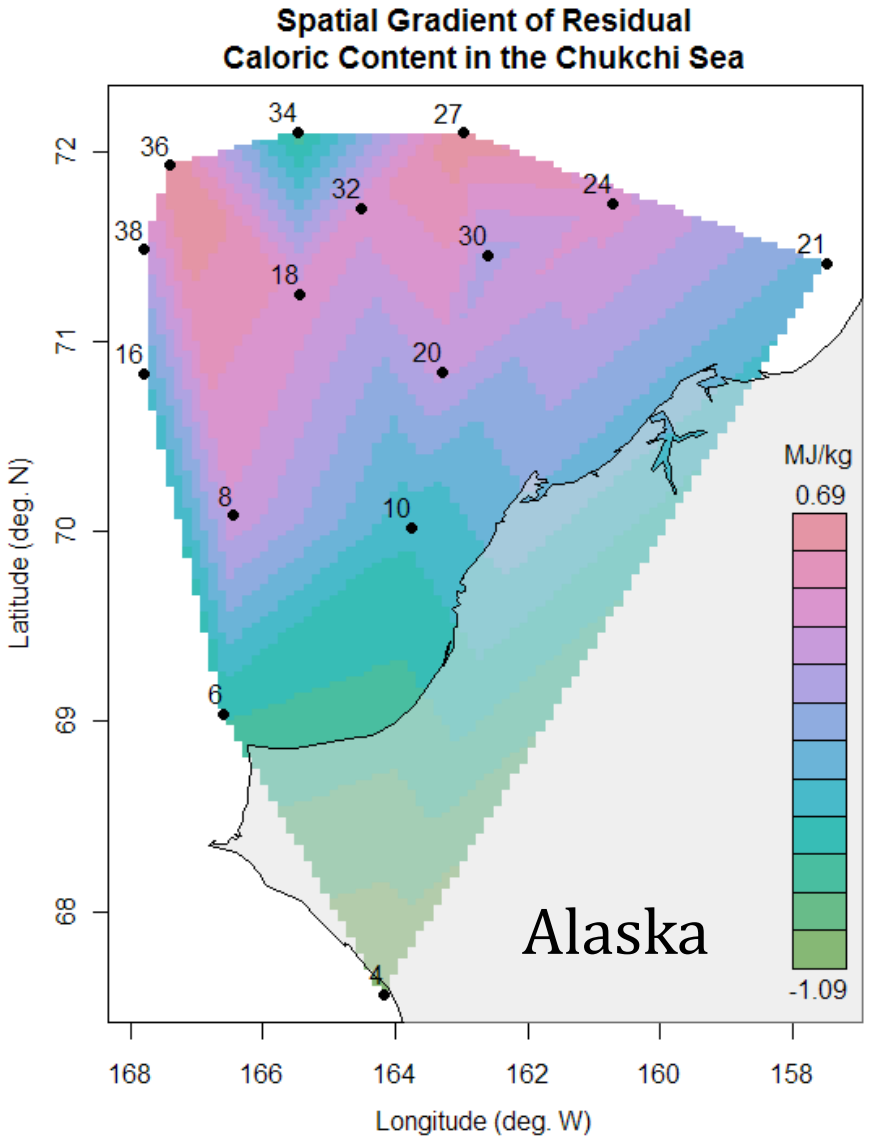

Figure 2.8: Spatially interpolated plot of residual caloric content (influence of taxonomic variables regressed out) for animals collected during the July-August 2010 COMIDA CAB cruise. Black dots are stations surveyed, with station number.

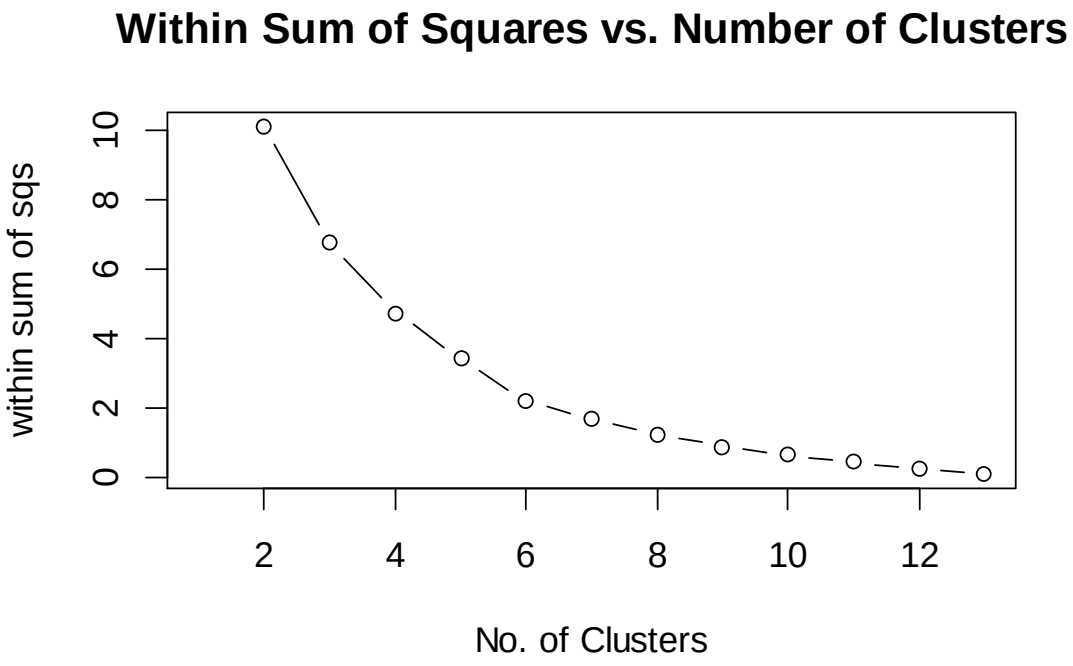

Figure 2.9: Plot of the within-groups sum of squares against number of clusters for the K-means cluster analysis. Animals were collected during the July-August 2010 COMIDA CAB cruise.

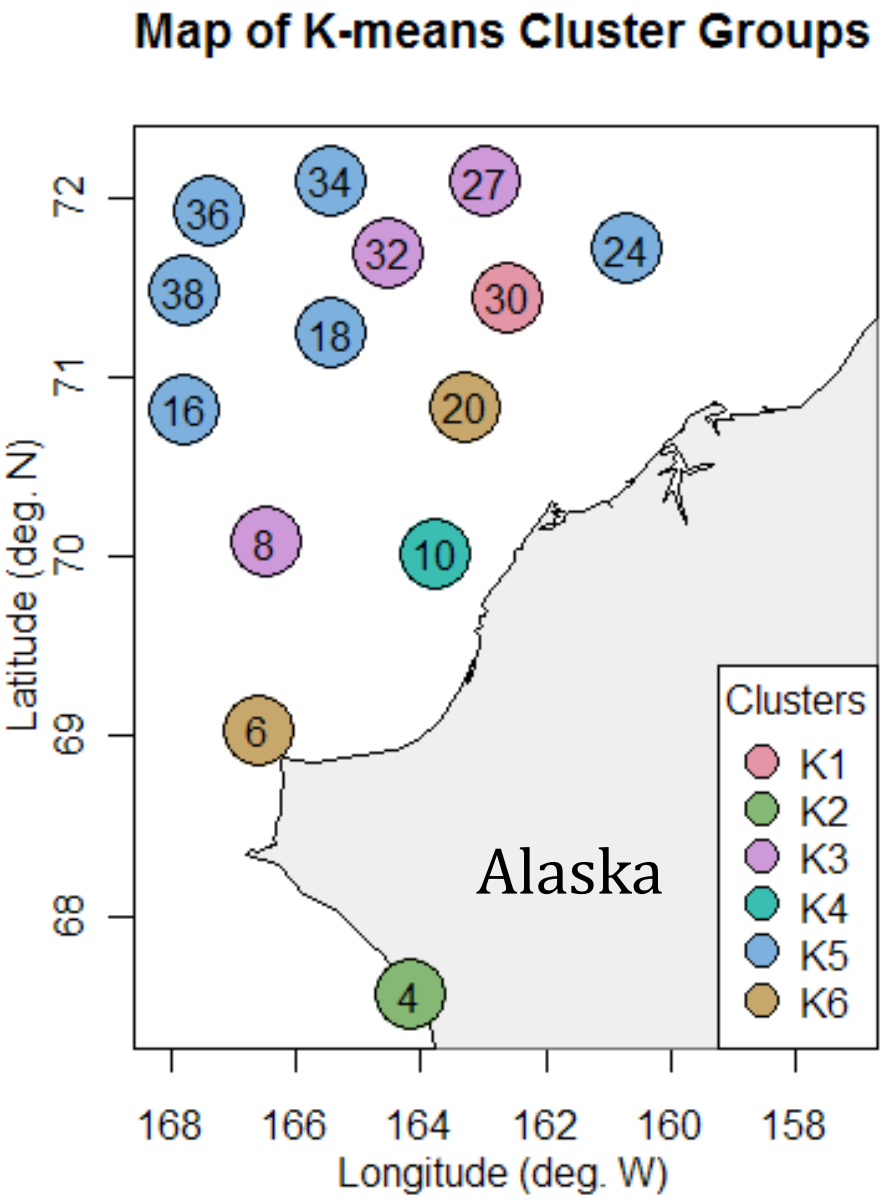

Figure 2.10: Map of the six cluster station groups produced by K-means cluster analysis. Animals were collected during the July-August 2010 COMIDA CAB cruise.

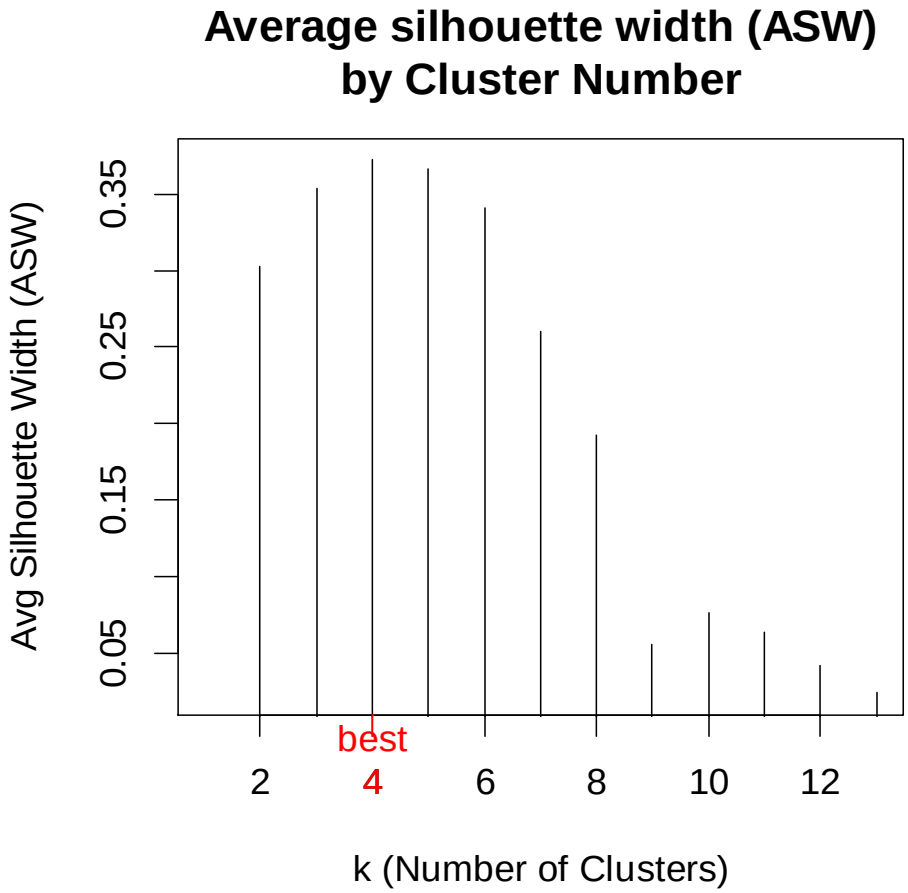

Figure 2.11: Average silhouette width (ASW) against number of clusters for the Partitioning Around Medioids (PAM) clustering method Animals were collected during the July-August 2010 COMIDA CAB cruise.

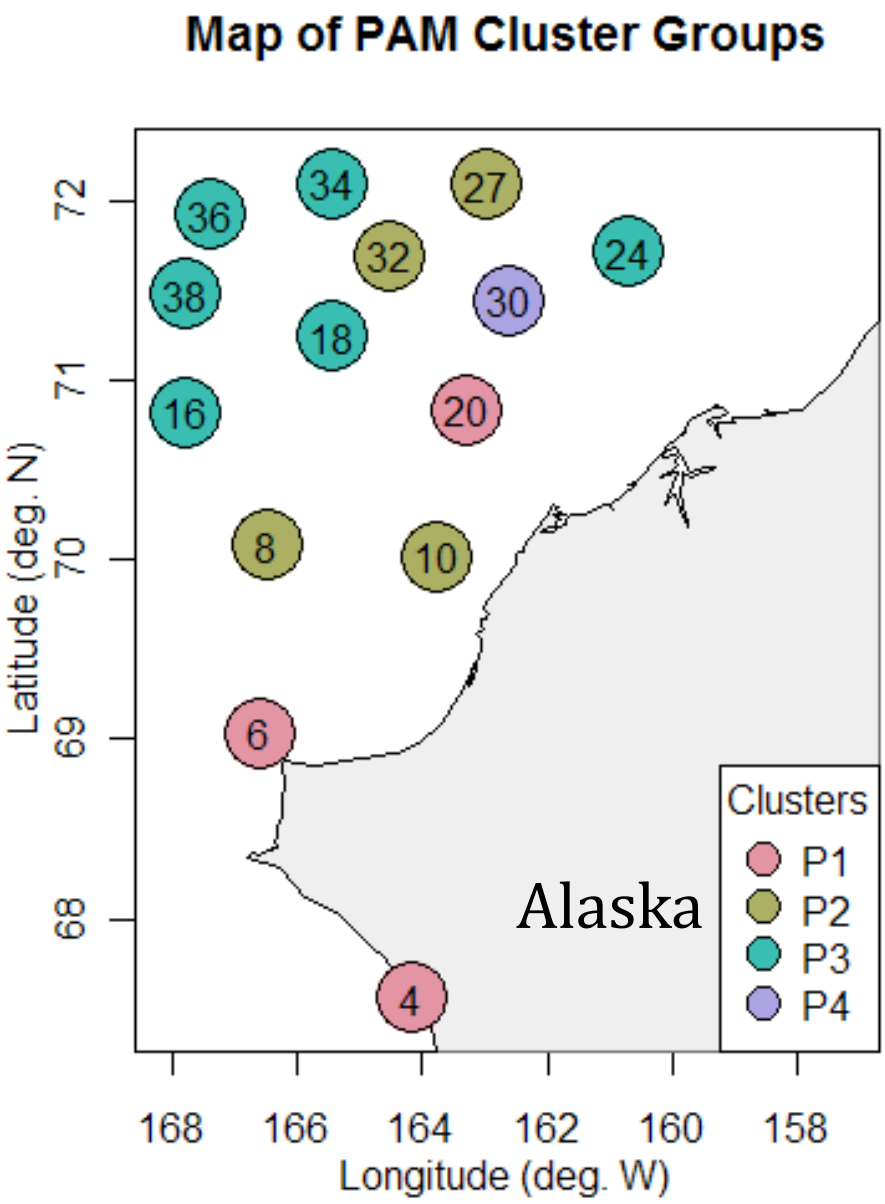

Figure 2.12: Map of the four cluster groups produced by Partitioning Around Medioids (PAM) cluster analysis, with 37% dissimilarity measured by average silhouette width (ASW). Animals were collected during the July-August 2010 COMIDA CAB cruise.

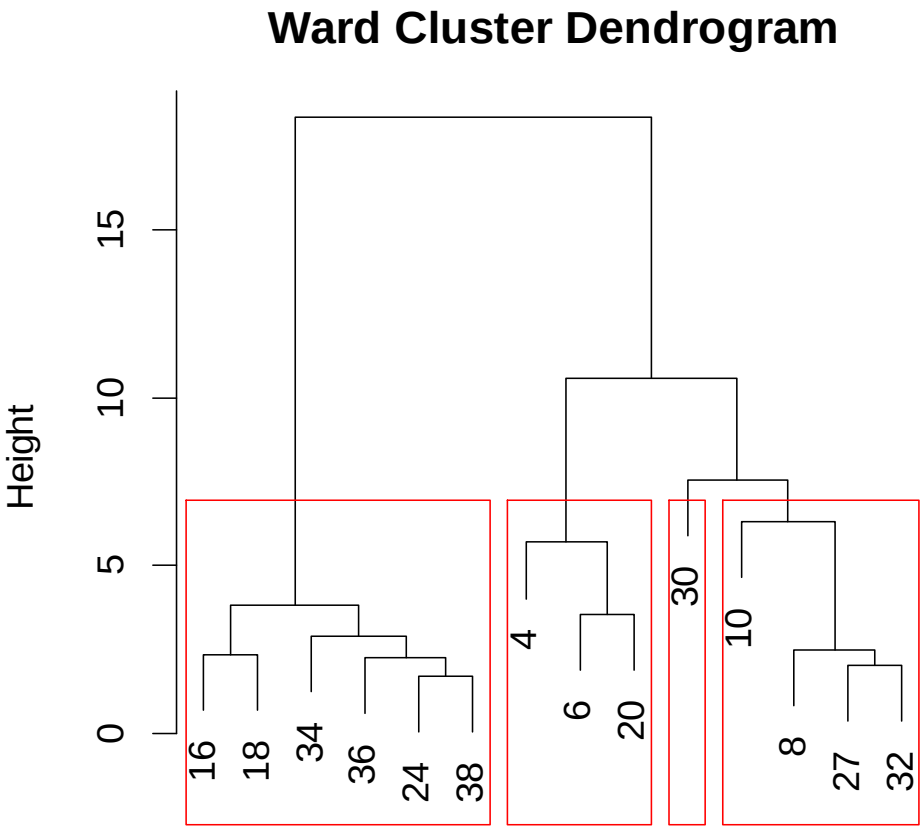

Figure 2.13: Dendrogram of hierarchical agglomerative approach to cluster analysis with 14 stations of caloric, spatial and environmental data using Ward's method. Red boxes surround the four identified cluster groups. Animals were collected during the July-August 2010 COMIDA CAB cruise.

Chapter 3: Caloric Density of Dominant Macroinvertebrate Fauna in the Pacific Arctic Region (PAR): Variation between Frozen and Formalin-preserved Samples

Abstract

Past and present caloric studies for benthic macroinvertebrates throughout the Arctic have employed a variety of preservation methods to animal tissue samples, including formalin fixation and freezing. These different preservation methods have led to variable caloric densities that are difficult to cross compare. In this investigation conducted in the Pacific Arctic Region (PAR), caloric energy contents were determined from frozen samples of 4 bivalve families, 9 polychaete families, 3 amphipod families, and 1 sipunculid family, and from formalin fixed samples of 4 bivalve families, 10 polychaete families, 4 amphipod families, and 1 sipunculid family. Significant differences in caloric energy content were found to exist between classes under each preservation method, with bivalves measuring at consistently higher caloric densities than amphipods and polychaetes for both preservation methods. No significant differences in caloric content were found to exist by sea (northern Bering vs. Chukchi Sea) for either frozen or formalin-preserved samples. Paired t-tests on the differences in caloric content between formalin-preserved and frozen samples from 23 infaunal macroinvertebrate families indicated that formalin fixation significantly increases caloric measurements in comparison to frozen samples by 3.3%. Among polychaetes, paired t-tests on 13 paired caloric observations (formalin versus frozen) yielded signficant differences between preservation techniques, with formalin-preserved polychaetes measuring at higher caloric contents than frozen polychaetes. The same result was found for bivalves ($p=0.047$), though low observation numbers (7) required the use of a nonparametric Wilcoxon signed-rank test. While a low number of observations for amphipods (3) also required the use of a nonparametric Wilcoxon signed-rank test, the differences between formalin-preserved and frozen samples were found to be non-significant ($p>0.05$). Over all, these results suggest that investigators must take into account preservation method while planning and cross comparing benthic invertebrate caloric contents in ecological studies.

Introduction

Integrated Ecosystem Studies in the PAR

The Pacific Arctic Region (PAR) is characterized by regions of very high productivity and contains some of the world's highest faunal biomasses. In this region, the benthos plays a larger role on trophic interactions than in temperate zones (Grebmeier and Barry 1991; Grebmeier et al. 1995). The northern Bering and southern Chukchi Seas also experience intense seasonal pulses in phytoplankton production, known to be largely driven by spring ice melt and breakup (Smith and Sakshaug 1990; Gradinger 2009). Food webs in these regions tend to be short in trophic length, with direct assimilation of phytoplankton by a vast population of benthic macroinvertebrates, resulting in high trophic efficiency (Dunton et al. 1989; Grebmeier and Dunton 2000; Grebmeier et al. 2006a). These macroinfaunal biomass levels are as high as 150 g C m^{-2} in some areas (Grebmeier 2012), and a number of higher trophic level predators including bottom feeding fish, whales, seals, walrus and diving birds rely upon them as food sources (Fay et al. 1977; Lowry et al. 1980; Hazard and Lowry 1984; Highsmith and Coyle 1992; Lovvorn et al. 2003; Cui et al. 2009; Iken et al. 2010).

One of the first comprehensive PAR benthic ecosystem investigations found a total of 472 species at 176 stations, which included 292 genera and 16 phyla (Stoker 1978). More continuous ecosystem time series studies on the benthic community have been ongoing in the PAR since the 1970s, resulting in many years of compiled biomass data for most of the infaunal taxa in the area (Grebmeier et al. 2006a). While it is anticipated that recent sea ice retreat and warming of the northern Bering and Chukchi

Sea waters will continue (Grebmeier et al. 2010), the associated impacts of this environmental change upon these biological systems are not as clear. This uncertainty has prompted the Pacific Arctic Group (see http://pag.arcticportal.org) to initiate a new program called the Distributed Biological Observatory (DBO), an international collaboration designed to serve as a change detection array along a latitudinal gradient spanning from the northern Bering Sea to Barrow, Alaska. Initiated in 2010, the DBO integrates environmental, chemical and biological studies for both the water column and the benthos in the PAR, and will link these data to observations of higher trophic level predators (Grebmeier, 2012; see http://www.noaa.gov/dbo).

Caloric Studies in the Arctic and PAR

Over the past 40 years, caloric energy studies of various biological components within temperate ecosystems have been common, but fewer studies have been published for the Arctic and associated ecological zones. The majority of caloric energy surveys for benthic invertebrates that have been conducted in the Arctic have occurred in the European Arctic (Szaniawska and Wolowicz 1986; Weslawski et al. 2010). These cover a variety of organisms, including polychaetes, crustaceans, molluscs and tunicates. Investigators in the northern Atlantic and Atlantic Arctic have also undertaken studies of benthic faunal caloric energy content, including projects in New Brunswick, Canada (Tyler 1973), and Frobisher Bay, where energy values for 121 marine benthic invertebrates have been surveyed, with over 18 classes represented (Wacasey and Atkinson 1987). Hyperiid amphipods in this region were found to have especially high

lipid content, resulting in high caloric content values (Percy and Fife 1980). It is likely that differences in caloric content among other various taxonomic groups are also related to lipid content. In one Canadian survey, nuculanid bivalves (*Yoldia hyperborea*) contained approximately 25 mg lipid/g wet weight (polar and neutral), while nephtyid (*Nephthys ciliate*) and terebellid (*Artacama proboscidea)* polychaetes contained 16.3 and 12.5 mg lipid/g wet weight, respectively (Parrish et al. 1996).

Only a few caloric studies have occurred in the PAR. Stoker (1978) determined caloric values for 52 species of benthic infauna encompassing 5 classes, and found organic carbon and caloric content to be highly correlated. In this study, the caloric contents of formalin-preserved bivalves averaged 4.85±0.13 kcal/g, 3.60±0.76 kcal/g for polychaetes, and 5.22±0.24 kcal/g for amphipods (Stoker 1978). Another recent study by Hondolero et al. (2011) evaluated the caloric content of a subset of PAR faunal organisms, covering 18 epifaunal taxa and 6 infaunal taxa. These authors reported values for formalin-preserved benthic invertebrates ranging from 2.45-5.00 kcal/g.

Comparisons of Preservation Methods for Animal Tissues

Caloric measurements require field collections, preservation, and post-field processing before analyzing samples in a land-based laboratory. Although preservation in formalin is common (e.g. Stoker 1978), it is not known if this impacts apparent caloric energy values, relative to studies where samples were frozen (e.g. Percy and Fife 1980; Lawson et al. 1998).

There have been a few comparisons of caloric energy content for benthic invertebrates in the PAR using different preservation methods. In one comparison, 9 species of bivalves and 6 species from other miscellaneous taxa were surveyed, and it was found that formalin-preserved animals in all but 3 taxa (*Yoldia hyperborea, Rhachotropis aculeata, Nephtys sp*.) had higher caloric densities by dry weight than those preserved by freezing (Stoker 1978). A more recent comparison reported that formalin preservation significantly increased the caloric value of only three out of seven taxa tested by Mann Whitney test, including a decapod (*Argis lar*) ($p=0.013$), and two anthozoans ($p=0.046$ and 0.050), though low sample size may have obscured significant differences (Hondolero et al. 2011).

In comparative studies between the various preservation methods available for animal tissues collected at sea, freezing is typically used as a control. The assumption that no change in caloric value occurs as a result of freezing has been called into question, because freezing results in the mechanical breakdown of cells as ice crystals form in the tissues, resulting in a possible loss of carbon (Benedito-Cecilio and Morimoto 2002; Feuchtmayr and Gray 2003). It is usually not practical to perform calorimetry measurements upon fresh samples while still at sea, but shock freezing may be a better alternative, as faster freezing times limit the formation of the ice crystals that damage tissue cells (Feuchtmayr and Gray 2003).

Objectives of Study

One objective of this study was to report current caloric energy values for frozen and formalin-preserved dominant taxa (by biomass) in the PAR. These values can serve as a source for future caloric investigations by region, or as a source for comparisons of caloric content within the PAR over time. The second objective of this study was to compare the effects of two preservation methods (formalin fixation and freezing) on the apparent caloric energy content of prey organisms collected in the Bering and Chukchi Seas. These data may aid in determining whether preservation method is important to take into consideration in caloric studies. Assuming that formalin fixing causes a change in the apparent caloric content relative to frozen samples generates a third and final objective: to generate conversion factors for converting wet weight formalin-preserved biomass to kilocalories. This conversion factor would allow for the conversion of decades of wet weight biomass data in the PAR to energy terms and allow for much broader scale comparisons of caloric content spatially and temporally, which would be informative for predator-prey studies.

Statements of Hypotheses

Hypothesis 3.1: There is no significant difference among organisms in the same taxon preserved by freezing or formalin.

Alternative Hypothesis: Because of differences in lipid content, significant differences in caloric content exist among bivalves, amphipods and polychaetes.

Hypothesis 3.2: Caloric content of benthic infauna collected from the Chukchi Sea will not significantly differ from benthic macroinvertebrates collected in the northern Bering Sea.

Alternative Hypothesis: Significant differences in the caloric content of benthic macroinvertebrates exist between the northern Bering and Chukchi Seas.

Hypothesis 3.3: There is no significant difference between the caloric values of benthic infaunal tissues preserved in formalin versus frozen samples.

Alternative Hypothesis: Formalin-preserved samples will have significantly higher caloric densities than frozen infaunal samples because formalin preservation adds carbon to tissues.

Methods

Sample Collection and Preparation

Benthic animals were collected between July 8th and July 20th, 2011 aboard the CCGS (Canadian Coast Guard Ship) Sir Wilfrid Laurier as part of the C30 project. The 18 sampling stations for this cruise were selected in 2010 as a part of the new DBO project (Grebmeier 2012) (Table 3.1; Figure 3.1).

At each of the stations where animals were collected for this project, two grabs were taken using a weighted 0.1 m^2 van Veen and sieved through a 1 mm screen. The animals in the first grab were preserved in 10% buffered seawater formalin, packaged, and returned to Chesapeake Biological Laboratory (CBL) in Maryland for post cruise processing. The second grab sample was sorted shipboard to the family level with a

dissecting microscope. Previously determined dominant infauna by biomass from prior cruises were used to identify faunal types that are potential walrus prey. These infaunal taxa included the following: bivalve families Tellinidae, Cardiidae, Nuculidae, Nuculanidae; polychaete families Ampharetidae, Capitellidae, Cirratulidae, Lumbrineridae, Maldanidae, Nephtyidae, Orbiniidae, Phyllodocidae, and Terebellidae; amphipod families Ampeliscidae, Isaeiidae, and Lysianassidae; and the sipunculid family Golfingia. After identification, animals were frozen by type in individual Whirl-pak® bags, and returned to CBL for post-cruise processing for caloric determinations. In addition, animals were separated by collection location from either the northern Bering Sea or Chukchi Sea.

Formalin-preserved grab samples were rinsed in freshwater and sorted at CBL under a dissecting microscope to the species level, or lowest taxonomic level possible. Infaunal family types were pooled together, separating the northern Bering from Chukchi Sea samples. For some infaunal species, there was not a sufficient amount by biomass for caloric analyses, so all the species within that family were combined into one aliquot and then analyzed. These samples included polychaetes from the families Capitellidae, Cirratulidae, and Orbiniidae.

Non-living materials were removed from samples prior to caloric analyses, specifically the calcium carbonate shells of all bivalves and the sediment tubes surrounding polychaetes, consistent with the methodology outlined in other caloric energy studies (see Wacasey and Atkinson 1987).

All frozen and formalin-preserved samples were weighed before placement in tins for desiccation in separate ovens at 80°C. They were weighed periodically over the

following days until constant weight was reached (typically in 5 to 6 days), indicating that a state of total desiccation necessary for calorimetry had been achieved. Dried samples were subsequently ground using a mechanical grinder into a homogeneous powder and stored in glass desiccators containing the desiccant DRIERITE®, and pelletized using a pellet press. For taxa with sufficient biomass to produce 1-3 g pellets, a large bomb calorimeter was used. For samples containing less than 2 g of sample, a semi-micro bomb was used and pellets weighed between 0.1-0.5 g. Some samples were too dry for pelletization, and were instead deposited into gel capsules. Exact pellet weights and gel capsule weights were recorded for each sample prior to calorimetry.

Calorimetry Procedures

To determine energy values, all pellets were combusted in a Parr Bomb Calorimeter (Model 6200). Caloric densities were measured in megajoules per kilogram (MJ/kg), and were corrected for the amount of fuse wire used and for the remaining amount of sample (see more detailed methods for caloric analysis in Chapter 2).

Calculation of Conversion Factors from Biomass to Caloric Content

In order to generate conversion values from infaunal wet weight to energy, select infaunal samples were sorted from van Veen grabs collected from on station (UTBS1) during the 2011 CCGS Sir Wilfrid Laurier cruise north of St. Lawrence Island as well as one station south of St. Lawrence Island (VNG1) collected the previous year aboard the

March 2010 Polar Sea cruise. Station VNG1 from the 2010 Polar Sea cruise is the same time series site as Station VNG1 (station #2) collected in July 2011 on the CCGS Sir Wilfrid Laurier (Figure 3.1). Bivalve shells and polychaete tubes were removed, and tissues were weighed before desiccation in an oven at 80° C. After constant weight was achieved, the new dry weight of each sample was measured for use in a conversion formula

$$r_w = dw/ww$$

where:

r_w = weight ratio of wet weight to dry weight

dw = dry weight

ww = wet weight

This factor, r_w, can be applied to estimate energy content from wet weight data for formalin-preserved infauna in the formula

$$E_{wwf} = E_{dwf} * r_w$$

where:

E_{wwf} = energy density of wet weight formalin-preserved benthic infauna

E_{dwf} = energy density of dry weight formalin-preserved benthic fauna or gel capsule corrected energy density of dry weight formalin-preserved benthic fauna

If E_{wwf} is multiplied by a wet weight for the same benthic organism that the dry weight caloric content and dry weight to wet weight ratio were calculated for, then a value in energy units would result. In this sense, E_{wwf} may be viewed as a conversion factor between wet weight and caloric energy.

The lowest matching northern Bering Sea formalin-preserved taxon available with a dry weight caloric value was used to calculate the wet weight caloric conversion factor in kcal/g wet.

Statistical Analysis

All statistical analyses were completed using R version 2.15.0 (see http://www.rproject.org and Appendix 1). While formalin-preserved animals were identified to the species level for the generation of conversion factors between wet weight and caloric energy, caloric measurements were averaged by family for statistical analyses. First, to statistically analyze for differences in energy content between infaunal classes for frozen and formalin-preserved samples from the Bering and Chukchi Seas, a one way Analysis of Variance (ANOVA) test was employed, and Homogeneity of Variance (HOV) and normality assumptions tested with Bartlett and Anderson Darling tests. Because there were not enough energy observations for families to satisfy

normality and HOV tests, a Kruskal-Wallis nonparametric test was applied as an alternative to one way ANOVA to look for significant differences in energy content between infaunal families throughout the northern Bering and Chukchi Seas. Differences between the regions (northern Bering and Chukchi Seas) were also investigated using a one way ANOVA test, with HOV and normality assumptions tested with Bartlett and Anderson Darling tests due to an increased n size to test using parametric statistics.

To statistically analyze for differences between frozen and formalin-preserved taxa in the northern Bering and Chukchi Seas, a non-parametric Wilcoxon signed-rank test was used because of low sample size in the cases of some classes of infauna. For those classes that satisfied normality tests, paired t-tests could also be used. All statistical analyses were evaluated at the 0.05 significance level. For all R packages used in this investigation and version numbers, see Appendix 1.

Results

Energy Differences by Preservation Technique Among Taxa

Energy content determinations were made for frozen samples from 5 bivalve families (Figure 3.2a), 9 polychaete families (Figure 3.3a), 3 amphipod families (Figure 3.4a), and 1 sipunculid family (Table 3.2). Energy content determinations were made for 4 formalin-preserved bivalve families (Figure 3.2b), 10 polychaete families (Figure 3.3b), 3 amphipod families (Figure 3.4b), and 1 sipunculid family (Table 3.3). The overall

mean energy content was 20.17±0.95. megajoules per kilogram (MJ/kg) for frozen samples, and 20.94±0.83 MJ/kg for formalin-preserved samples.

The average bivalve energy content was highest of all classes under both preservation methods, specifically 21.05±0.60 MJ/kg for frozen samples (range: 20.26 – 21.83 MJ/kg, n=8) and 21.67±0.79. MJ/kg for formalin-preserved samples (range: 20.05 – 22.69 MJ/kg, n=13). Amphipods and polychaetes measured at similar caloric levels. For frozen samples, the average caloric density measured for amphipods was 20.10±0.18 MJ/kg (range: 19.84 – 20.24 MJ/kg, n=4), and the polychaetes measured lower at an average of 19.92±0.49 MJ/kg (range: 19.10 – 20.90 MJ/kg n=15) (Figure 3.5a). When preserved in formalin, the average amphipod caloric content measured as 20.54±0.28 MJ/kg (range: 20.21 – 20.84 MJ/kg, n=4), while the average polychaete caloric content measured higher at 20.70±0.58 MJ/kg (range: 19.42 – 22.01 MJ/kg, n=24) (Figure 3.5b). The one sipunculid family represented (*Golfingia* sp.) measured 17.26 MJ/kg frozen compared to 18.87 MJ/kg formalin fixed. Because caloric determinations could only be made for one family, no statistical testing of the sipunculids could be accomplished.

Using class as the group descriptor, the frozen and formalin-preserved energy value data sets satisfied HOV testing (Bartlett test p-values of 0.156 for frozen, and 0.084 for formalin-preserved) and the residuals satisfied normality testing (Anderson-Darling test p-values of 0.892 for frozen, and 0.192 for formalin-preserved) with significance set at 0.05. A one way ANOVA analysis between frozen infaunal energy content and class resulted in a highly significant model ($r=0.548$; $p<0.001$; $n=25$), and a one way ANOVA analysis between formalin-preserved energy content and class resulted in another significant model ($r=0.327$; $p=0.007$; $n=28$).

Frozen and formalin-preserved energy values by infaunal family rather than class were also examined, but did not satisfy normality or HOV tests because of the low sample size within each family, and thus had to be tested using Kruskal-Wallis, a nonparametric alternative to the parametric one way ANOVA. Analyses on data generated from both frozen and formalin-preserved samples resulted in nonsignificant p-values ($p>0.05$).

Northern Bering versus Chukchi Seas

All energy content data satisfied HOV testing (frozen $p=0.530$; formalin $p=0.393$), and residuals passed HOV testing (frozen $p=0.065$; formalin $p=0.162$) for samples collected in the northern Bering and Chukchi Seas. A one way ANOVA was applied to all invertebrate caloric contents with data from each sea (northern Bering vs. Chukchi Sea) separated. This analysis yielded no significant differences in mean values between samples collected in the Bering and Chukchi Seas for the dataset of all observed caloric measurements (frozen $r<0.001$, $p=0.945$, $n=25$; formalin $r=0.007$, $p=0.683$, $n=28$).

Comparison of Preservation Methods

A total of 23 paired energy observations were made by family, with 7 paired bivalve comparisons, 13 paired polychaete comparisons, and 3 paired amphipod comparisons (Table 3.4). All differences between the paired frozen and formalin-preserved energy values were normally distributed (Anderson-Darling $p=0.820$), thus a

paired t-test was used to test for significant differences. A significant difference between frozen and formalin-preserved taxa was found ($p<0.001$), with formalin-preserved samples consistently having higher energy content than the frozen samples (Figure 3.6a). The overall percent increase in caloric content due to formalin fixation was 3.3%.

As there were only 7 paired observations for bivalves, normality tests could not be completed for the differences and the nonparametric comparison test Wilcoxon signed rank test had to be applied. The test resulted in a significant ($p=0.047$, $n=7$) difference between frozen and formalin-preserved samples, with formalin-preserved bivalves having a higher caloric density than frozen samples (Figure 3.6b).

The differences between the 13 paired energy values for polychaetes alone satisfied normality tests (Anderson-Darling $p=0.118$, $n=13$), so a parametric paired t-test was subsequently used to compare preservation methods. As before, the results of the t-test ($p<0.001$, $n=13$) suggested that significant difference exists between the frozen and formalin-preserved samples, with formalin samples consistently measuring at higher energy densities than frozen samples (Figure 6c).

Because only 3 paired observations were made for amphipods, a Wilcoxon signed rank test was again used because normality tests could not be applied to the differences between the caloric contents of amphipods under each preservation method. In this case the test resulted in a nonsignificant ($p>0.05$, $n=3$) difference between frozen and formalin-preserved samples (Figure 6d).

Conversion Values

The comparative Bering-Chukchi Sea analysis included 32 wet weight to kilocalorie conversions that could be calculated, including 15 amphipod taxa, 13 polychaete taxa, and 4 bivalve taxa (Table 3.5). Dry weight to wet weight ratios ranged from 0.031 to 0.537, representing a range of approximately 46 – 97% reduction in weight amongst benthic macroinvertebrate taxa to reach a state of complete desiccation after formalin preservation. Wet weight caloric conversion factors ranged from 0.15 to 2.62 kcal/g wet.

Discussion

Comparisons of Energy Measurements to Previous Studies

The caloric densities determined are similar to reported literature values from the eastern Canadian and European Arctic. In the Canadian Arctic, fresh bivalve meat from some of the same faunal families evaluated had energy densities ranging from 2.61 to 4.89 kcal/g. Polychaetes in that study ranged from 1.079 to 5.13 kcal/g, amphipods ranged from 3.076 to 3.96 kcal/g, and sipunculids of the same genus as in this study (*Golfingia*) measured 3.11 kcal/g (Wacasey and Atkinson 1987). Interestingly, their reported energy per unit weight is significantly less than the lowest 4.12 kcal/g energy density measured for frozen sipunculids in this study.

The recent Hondolero et al. (2011) caloric study of analyzed infauna from the same geographic area presented here. This work determined that energetic densities for frozen samples of bivalves were a mean of 4.77 kcal/g and 4.42 kcal/g for the families

Nuculidae and Tellinidae, respectively. There were variable results in that investigation for formalin-preserved bivalves, specifically 5.45 and 4.92 kcal/g from the families Cardiidae and Tellinidae, respectively (Hondolero et al. 2011). By comparison, averaged caloric values for samples of those same bivalves in this study had higher average values for frozen samples, specifically 4.89 kcal/g for nuculid bivalves and 5.15 kcal/g for tellinid bivalves. Formalin-preserved cardiid bivalves in this study averaged 5.009 kcal/g and tellinid bivalves averaged 5.23 kcal/g. Few polychaete worms were sampled by Hondolero et al. (2011), but formalin-preserved caloric content from nephtyid polychaetes averaged 3.77 kcal/g (Hondolero et al. 2011), lower than the average 4.99 kcal/g for formalin-preserved nephtyid polychaetes in this investigation.

The energy content of formalin-preserved bivalves reported during a 1973-77 survey in the Bering and Chukchi Seas was 4.85 kcal/g (range: 4.46 – 5.14 kcal/g), with polychaetes averaging 3.60 kcal/g (range: 1.40 – 5.60 kcal/g), amphipods averaging 5.22 kcal/g (range: 4.71 – 6.040 kcal/g), and sipunculids with an average value of 3.01 kcal/g (Stoker 1978). In this current investigation, caloric densities for formalin-preserved bivalves, polychaetes and the one family of sipunculid (means of 5.18, 4.95, and 4.51 kcal/g, respectively) had higher caloric energy contents than the values reported by Stoker et al. (1978). By contrast, amphipods in this study measured lower than Stoker's formalin-preserved amphipods of 4.91 kcal/g, perhaps due to sampling season. Although our study was not designed to look at decadal variability in faunal energy values, Hondolero (2011) did evaluate changes in energy content in the Bering and Chukchi Seas over time, and found that most present day energy values were not statistically different from historic ones. However, 2 out of 7 faunal comparisons did

suggest that energy content increased for two epibenthic taxa (*Hyas coarctatus* and *Gersemia rubiformis*).

Discrepancies between published energy densities may be related to a number of causes. Sampling procedures in the field are one factor that may largely affect caloric values. Differences in location, sampling season, and year could yield large differences in species collected, their size, health, and lipid content. In Greenland, it has been demonstrated that significant variability in lipid and protein content exist for two species of bivalves (families Astartidae and Propeamussiidae) over east/west spatial gradients (Gallagher et al. 1998). Seasonal changes in lipid content related to reproductive events (Mann, 1978; Okumus and Stirling 1998) can also have profound effects on faunal caloric results (Tyler 1973). Studies of multiple species of amphipods in the European Arctic indicate no statistically significant differences in energy for samples collected during various seasons due to nearly stable environmental conditions throughout the year (Szaniawska and Wolowicz 1986). However, by comparison, the PAR is characterized by strong seasonality with variable ice cover and primary production (Grebmeier et al. 1995). Ecosystem changes on larger temporal scales are also occurring in the PAR, such as changing sea ice extent and duration, and seawater warming that can potentially influence both species composition and benthic community structure. An ecosystem shift from benthic- to pelagic-dominated communities has been suggested as a response to these environmental changes in the northern Bering Sea (Grebmeier et al. 2006b), which could potentially alter community caloric energy densities of benthic invertebrate communities over time.

In addition to field sampling, differences in sample processing may also cause discrepancies between caloric energy values for different animals. These differences could include using different formalin to seawater concentrations for formalin preservation, using different temperatures for freezing, selecting different tissues for calorimetry, and even the use of various types of calorimeters and instruments during calorimetry itself. For example, Hondolero et al. (2011) used a 4% buffered formaldehyde-seawater solution for preservation, in contrast to the 10% buffered seawater used in this study. These differences highlight the importance of not only reporting all aspects of processing methods used for an investigation, but also the need for standardization across the field. This will allow for easier cross comparisons to be made.

Energy Comparisons by Taxa and Sea

The first alternative hypothesis tested in this chapter was that bivalves and amphipods have higher caloric densities than polychaetes. In this case, the data has yielded complex results. While bivalves consistently measured at higher caloric densities than amphipods and polychaetes under both preservation methods, amphipods and polychaetes measured at similar values, with the amphipod mean caloric content higher than the polychaete mean for frozen samples, and the polychaete mean caloric content higher than the amphipod mean for formalin samples. Supporting these high bivalve caloric values, other caloric surveys have also reported similar results in different regions of the Arctic (Stoker 1978; Wacasey and Atkinson 1987; Hondolero et al. 2011).

Bivalves contain high lipid levels (Parrish et al. 1996), which likely explains the higher caloric contents for these organisms, and their roles as preferred food items for higher trophic level organisms such as walrus, seals, and eider ducks (Fay et al. 1977; Lowry et al. 1980; Lovvorn et al. 2003). Walrus in particular, while known to consume a wide variety of benthic invertebrates, prefer softer bodied organisms that are higher in fat content. During studies of almost 800 Pacific walruses (*Odobenus rosmarus divergens*), bivalves occurred most frequently among prey taxa in walrus stomachs from the Bering Sea, while gastropods dominated for the Chukchi Sea (Sheffield and Grebmeier 2009). Despite the fact that sipunculids were found in this current study to be lower than bivalves and polychaetes in caloric density, similar to results found from other infaunal caloric energy surveys (Stoker 1978; Wacasey and Atkinson 1987), these infauna are common food items for walrus, appearing in one third of walrus stomachs analyzed in the Bering and Chukchi Seas (Sheffield and Grebmeier 2009). The calculated conversion factors to convert wet weight biomass to kilocalories will be particularly useful to further investigate predator prey relationships on broader spatial and temporal scales.

The second hypothesis of this chapter tested was that the caloric content of infaunal macroinvertebrates collected from the Chukchi Sea would not significantly differ from infaunal macroinvertebrates collected the northern Bering Sea. The data supports this hypothesis. Because the ANOVA did not screen out the influence of taxon upon caloric content, the findings reflected the taxonomic groups found in the area. For example, the Chukchi Sea study area contains high numbers of lipid rich bivalves, and the Bering Sea study area contains zones with lipid-rich amphipods as well as bivalves

(Grebmeier 2012). For a more comprehensive spatial analysis of energy content within the PAR, including a screen for taxonomic influence, refer to chapter 2 of this thesis.

Comparison of Formalin vs. Frozen

It has been hypothesized in the literature that a loss of carbon by cell lysis occurs as animal tissues are frozen (Feuchtmayr and Grey 2003). Unfortunately, there is a lack of published reports that quantify what this loss of carbon may be, and determine if it is significant. Due to the unavailability of calorimetric equipment at sea, we were unable to conduct calorimetry upon fresh animal tissues, and instead had to use frozen samples as a control.

The third alternative hypothesis of this thesis was that infaunal macroinvertebrate tissues preserved in formalin yield higher caloric densities than frozen samples. This alternative hypothesis is confirmed using all of the 23 paired observations at the family level. There is a significant difference in caloric energy content between animal tissue samples preserved in formalin and those preserved by freezing using a paired t-test on all 23 paired observations from all taxa. While paired comparisons within taxonomic classes alone resulted in significant differences between frozen and formalin-preserved polychaetes and bivalves, the paired observations within classes did not always support this hypothesis. For example, amphipod caloric content did not significantly vary between the preservation methods. This may be due to low sample size. With only 3 families, the amphipods had the lowest number of observations within any class (polychaete n=13; bivalve n=7).

The finding that the caloric content of formalin-preserved samples differ from frozen samples using all paired observations is supported by results from previous studies (Stoker 1978; Hondolero et al. 2011). This result may occur because the chemical characteristics of preserved animal tissues exhibit characteristics of their preserving chemicals. Once fresh tissue samples are immersed in formalin or formaldehyde, their isotopic signatures are known to shift toward the signatures of the preservative (Hobson et al. 1997). Though formalin is known to hydrolyze proteins (Von endt 1994; Hobson et al. 1997), it also directly contributes carbon, as it is a carbon based chemical (Feuchtmayr and Grey 2003). This addition of carbon to the total carbon mass is likely responsible for the higher energy densities noted for formalin-preserved benthic samples in comparison to frozen samples.

Conclusions

As with other comparisons of energy content among tissues preserved with varying methods, this study is limited by small sample size. These data indicate that energy densities measured from formalin-preserved faunal samples are statistically higher than those energy values measured for frozen samples. This is a finding that should be noted by any investigators planning to conduct caloric studies. It also serves as a warning against cross comparing literature caloric values without considering differences in sample preservation and the seasonality of sample collection, though the question remains of whether or not the percent increase due to formalin- fixation (3.3%) is large enough to consider important. Future comparative studies require an increase in

replicates of specific foci taxa, along with providing for standardized seasonal measurements to reduce growth and seasonality impacts on the individual organisms. These efforts will facilitate a better understanding of the factors influencing caloric energy content of prey organisms. These future studies are also necessary to understand energy availability of prey and associate energy flow through ecosystems.

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Tables

Table 3.1: Sampling stations, associated descriptions and environmental parameters for caloric content measurements taken during the July 2011 CCGS Sir Wilfrid Laurier cruise (SWL), ordered by station number.

Station Number	Station Name	Collection Date (mm/dd/yyyy)	Sea	Latitude (°N)	Longitude (°W)	Depth (m)	Bottom water Temperature (°C)	Bottom water Salinity (psu)
02	SLIP1	07/15/2011	Bering	62.010	175.060	80	-0.0574	32.32
03	SLIP2	07/15/2011	Bering	62.050	175.210	80	-0.1288	32.29
04	SLIP3	07/15/2011	Bering	62.390	174.570	68	-1.4914	32.02
05	SLIP5	07/15/2011	Bering	62.560	173.551	65	-1.4451	32.11
06	SLIP4	07/15/2011	Bering	63.030	173.460	71	-0.0726	32.63
013	UTN1	07/17/2011	Chukchi	66.710	168.400	35	6.2411	31.02
014	UTN2	07/17/2011	Chukchi	67.050	168.729	45	3.8327	31.72
015	UTN3	07/17/2011	Chukchi	67.330	168.909	49	3.9666	32.07
016	UTN4	07/17/2011	Chukchi	67.500	168.908	49	2.9296	32.51
017	UTN5	07/17/2011	Chukchi	67.670	168.910	50	2.8896	32.46
018	UTN6	07/18/2011	Chukchi	67.740	168.440	49	3.5616	32.13
019	SEC2	07/18/2011	Chukchi	67.780	168.600	50	3.4149	32.23
020	UTN7	07/18/2011	Chukchi	68.000	168.910	57	2.9702	32.71
021	SEC3	07/18/2011	Chukchi	67.900	168.240	58	3.3223	32.27
022	SEC4	07/18/2011	Chukchi	68.010	167.871	52	3.5379	32.11
024	SEC6	07/18/2011	Chukchi	68.190	167.311	47	4.0568	31.82
025	SEC7	07/18/2011	Chukchi	68.240	167.121	43	4.7871	31.43
026	SEC8	07/18/2011	Chukchi	68.301	166.942	34	6.6851	31.23

Table 3.2: Caloric densities (MJ/kg and kcal/g) determined for all Bering and Chukchi Sea frozen taxa collected during the July 2011 CCGS SWL cruise.

Sea	Class	Family	Caloric Content (MJ/kg)	Caloric Content (kcal/g)
Bering	Amphipoda	Ampeliscidae	19.839	4.742
Bering	Amphipoda	Ampeliscidae	20.135	4.812
Bering	Amphipoda	Isaeidae	20.239	4.837
Bering	Amphipoda	Lysianassidae	20.187	4.825
Bering	Bivalvia	Nuculanidae	21.363	5.106
Bering	Bivalvia	Nuculidae	20.645	4.934
Bering	Bivalvia	Tellinidae	21.464	5.130
Bering	Polychaeta	Ampharetidae	19.605	4.686
Bering	Polychaeta	Maldanidae	19.989	4.778
Bering	Polychaeta	Nephtyidae	19.875	4.750
Bering	Polychaeta	Orbiniidae	19.861	4.747
Bering	Polychaeta	Phyllodocidae	19.106	4.567
Bering	Polychaeta	Phyllodocidae	20.074	4.798
Bering	Polychaeta	Terebellidae	20.895	4.994
Chukchi	Bivalvia	Astartidae	20.969	5.012
Chukchi	Bivalvia	Cardiidae	20.256	4.841
Chukchi	Bivalvia	Nuculanidae	21.832	5.218
Chukchi	Bivalvia	Nuculidae	20.307	4.854
Chukchi	Bivalvia	Tellinidae	21.591	5.160
Chukchi	Polychaeta	Ampharetidae	20.356	4.865
Chukchi	Polychaeta	Capitellidae	20.243	4.838
Chukchi	Polychaeta	Cirratulidae	20.291	4.850
Chukchi	Polychaeta	Lumbrineridae	19.098	4.564
Chukchi	Polychaeta	Maldanidae	20.027	4.787
Chukchi	Polychaeta	Nephtyidae	19.351	4.625
Chukchi	Polychaeta	Orbiniidae	19.714	4.712
Chukchi	Polychaeta	Phyllodocidae	20.233	4.836
Chukchi	Sipunculidea	Golfingiidae	17.256	4.124
		Mean	20.172	4.821
		Standard Deviation	0.896	0.214

Table 3.3: Caloric densities (MJ/kg and kcal/g) determined for all Bering and Chukchi Sea formalin-preserved taxa collected during the July 2011 CCGS SWL cruise. Taxa are identified to species where possible.

Sea	Class	Family	Species	Caloric Content (MJ/kg)	Caloric Content (kcal/g)
Bering	Amphipoda	Ampeliscidae	*Ampelisca sp.*	20.844	4.982
Bering	Amphipoda	Ampeliscidae	*Byblis sp.*	20.213	4.831
Bering	Amphipoda	Isaeidae	*Protomedeia sp.*	20.669	4.940
Bering	Amphipoda	Lysianassidae	*Anonyx sp.*	20.434	4.884
Bering	Bivalvia	Cardiidae	*Serripes groenlandicus*	20.046	4.791
Bering	Bivalvia	Nuculanidae	*Nuculana pernula*	22.449	5.365
Bering	Bivalvia	Nuculidae	*Ennucula tenuis*	21.641	5.172
Bering	Bivalvia	Nuculidae	*Nucula nucleas*	20.553	4.912
Bering	Bivalvia	Tellinidae	*Macoma calcarea*	22.533	5.385
Bering	Bivalvia	Tellinidae	*Macoma moesta*	21.393	5.113
Bering	Bivalvia	Tellinidae	*Macoma torelli*	20.98	5.014
Bering	Polychaeta	Capitellidae	*sp.*	20.333	4.860
Bering	Polychaeta	Cirratulidae	*sp.*	20.491	4.897
Bering	Polychaeta	Lumbrineridae	*Lumbrineris sp.*	20.849	4.983
Bering	Polychaeta	Maldanidae	*Axiothella catenata*	21.096	5.042
Bering	Polychaeta	Maldanidae	*Maldane sarsi*	21.01	5.022
Bering	Polychaeta	Maldanidae	*Praxiella praetermissa*	20.9	4.995
Bering	Polychaeta	Nephtyidae	*Nephtys ciliata*	20.792	4.970
Bering	Polychaeta	Nephtyidae	*Nephtys punctata*	21.283	5.087
Bering	Polychaeta	Orbiniidae	*Leitoscoloplos pugattensis*	20.502	4.900
Bering	Polychaeta	Orbiniidae	*Scoloplos armiger*	20.897	4.995
Bering	Polychaeta	Phyllodocidae	*Eteone auricanta*	20.464	4.891
Bering	Polychaeta	Terebellidae	*Artacama proboscidea*	22.012	5.261
Chukchi	Bivalvia	Cardiidae	*Serripes sp.*	21.873	5.228
Chukchi	Bivalvia	Nuculanidae	*Yoldia hyperborea*	21.478	5.133

Chukchi	Bivalvia	Nuculidae	*Ennucula tenuis*	22.205	5.307
Chukchi	Bivalvia	Tellinidae	*Macoma calcarea*	22.119	5.287
Chukchi	Bivalvia	Tellinidae	*Macoma moesta*	21.692	5.185
Chukchi	Bivalvia	Tellinidae	*Tellina lutea*	22.688	5.423
Chukchi	Polychaeta	Ampharetidae	*Ampharete lindstroemi*	21.752	5.199
Chukchi	Polychaeta	Capitellidae	*sp.*	21.23	5.074
Chukchi	Polychaeta	Cirratulidae	*sp.*	20.85	4.983
Chukchi	Polychaeta	Lumbrineridae	*Lumbrineris sp.*	20.553	4.912
Chukchi	Polychaeta	Maldanidae	*Nichomache sp.*	19.643	4.695
Chukchi	Polychaeta	Maldanidae	*Praxiella gracilis*	20.126	4.810
Chukchi	Polychaeta	Maldanidae	*Praxiella praetermissa*	20.653	4.936
Chukchi	Polychaeta	Maldanidae	*Rhodine glaciolor*	20.097	4.803
Chukchi	Polychaeta	Nephtyidae	*Nephtys punctata*	20.566	4.915
Chukchi	Polychaeta	Orbiniidae	*sp.*	20.823	4.977
Chukchi	Polychaeta	Pectinariidae	*Pectinaria granulata*	19.417	4.641
Chukchi	Polychaeta	Phyllodocidae	*Eteone sp.*	20.394	4.874
Chukchi	Sipunculidea	Golfingiidae	*Golfingia sp.*	18.871	4.510
			Mean	20.938	5.004
			Standard Deviation	0.834	0.200

Table 3.4: Paired energy observations (MJ/kg) for dominant benthic macroinvertebrate fauna in the Pacific Arctic Region (PAR) and associated differences between formalin energy content and frozen energy content). Animals were collected during the July 2011 CCGS SWL cruise.

Sea	Class	Family	Formalin-preserved Energy (MJ/kg)	Frozen Energy (MJ/kg)	Differences (MJ/kg)
Bering	Amphipoda	Ampeliscidae	20.528	19.987	0.541
Bering	Amphipoda	Isaeidae	20.669	20.239	0.430
Bering	Amphipoda	Lysianassidae	20.434	20.187	0.246
		Class Mean	20.544	20.138	0.406
Bering	Bivalvia	Nuculanidae	22.449	21.363	1.086
Bering	Bivalvia	Nuculidae	21.097	20.645	0.452
Bering	Bivalvia	Tellinidae	21.635	21.464	0.171
Chukchi	Bivalvia	Cardiidae	21.873	20.256	1.617
Chukchi	Bivalvia	Nuculanidae	21.478	21.832	-0.354
Chukchi	Bivalvia	Nuculidae	22.205	20.307	1.898
Chukchi	Bivalvia	Tellinidae	22.167	21.591	0.576
		Class Mean	21.843	21.065	0.778
Bering	Polychaeta	Maldanidae	21.002	19.989	1.013
Bering	Polychaeta	Nephtyidae	21.037	19.875	1.163
Bering	Polychaeta	Orbiniidae	20.699	19.861	0.839
Bering	Polychaeta	Phyllodocidae	20.464	19.590	0.874
Bering	Polychaeta	Terebellidae	22.012	20.895	1.118
Chukchi	Polychaeta	Ampharetidae	21.752	20.356	1.396
Chukchi	Polychaeta	Capitellidae	21.230	20.243	0.987
Chukchi	Polychaeta	Cirratulidae	20.850	20.291	0.559
Chukchi	Polychaeta	Lumbrineridae	20.553	19.098	1.456

Chukchi	Polychaeta	Maldanidae	20.130	20.027	0.102
Chukchi	Polychaeta	Nephtyidae	20.566	19.351	1.215
Chukchi	Polychaeta	Orbiniidae	20.823	19.714	1.110
Chukchi	Polychaeta	Phyllodocidae	20.394	20.233	0.161
		Class Mean	20.886	19.963	0.922

Table 3.5: Dry weight to wet weight ratios and conversion factors (kcal/g wet) to caloric energy units for all species identified from the Bering Sea (SWL11 = CCGS SWL 2011 cruise, station UTBS1; PS2010=USCGC Polar Sea 2010 cruise, station VNG1). All dry weight caloric contents are given at the lowest matching taxon for Bering Sea only.

Class	Family	Species	Cruise	Wet Weight (g)	Dry Weight (g)	Dry Weight to Wet Weight Ratio	Lowest Matching Taxon With Caloric Measurement	Dry Weight Caloric Content (kcal/g dry)	Wet Weight Caloric Conversion Factor (kcal/g wet)
Amphipoda	Ampeliscidae	*Ampelisca birulai*	SWL11	0.011	0.002	0.143	*Ampelisca sp.*	4.982	0.712
Amphipoda	Ampeliscidae	*Ampelisca erythrorhabdota*	SWL11	0.300	0.036	0.119	*Ampelisca sp.*	4.982	0.592
Amphipoda	Ampeliscidae	*Ampelisca eschriti*	SWL11	1.407	0.142	0.101	*Ampelisca sp.*	4.982	0.504
Amphipoda	Ampeliscidae	*Ampelisca macrocephalia*	SWL11	10.746	1.228	0.114	*Ampelisca sp.*	4.982	0.569
Amphipoda	Ampeliscidae	*Byblis sp.*	SWL11	3.150	0.321	0.102	*Ampelisca sp.*	4.831	0.493
Amphipoda	Isaeidae	*Photis spasskii*	SWL11	0.034	0.004	0.104	Isaeidae	4.837	0.505
Amphipoda	Isaeidae	*Photis vinogradovi*	SWL11	0.006	0.001	0.194	Isaeidae	4.837	0.936
Amphipoda	Isaeidae	*Protomedeia fasciate*	SWL11	1.522	0.118	0.077	*Protomedeia sp.*	4.940	0.381
Amphipoda	Isaeidae	*Protomedeia grandimana*	SWL11	2.427	0.145	0.060	*Protomedeia sp.*	4.940	0.295
Amphipoda	Isaeidae	*Protomedeia popovi*	SWL11	0.329	0.024	0.074	*Protomedeia sp.*	4.940	0.366
Amphipoda	Isaeidae	*Protomedeia sp.*	SWL11	0.525	0.037	0.071	*Protomedeia sp.*	4.940	0.349
Amphipoda	Lysianassidae	*Anonyx sp.*	SWL11	0.174	0.094	0.537	Lysianassidae	4.884	2.621
Amphipoda	Lysianassidae	*Centromedon sp.*	SWL11	0.006	0.000	0.031	Lysianassidae	4.884	0.153
Amphipoda	Lysianassidae	*Onisimus sp.*	SWL11	0.009	0.001	0.141	Lysianassidae	4.884	0.690
Amphipoda	Lysianassidae	*Orchomeme sp.*	SWL11	0.078	0.013	0.166	Lysianassidae	4.884	0.813
Bivalvia	Nuculanidae	*Nuculana radiata*	PSEA10	0.610	0.099	0.162	Nuculanidae	5.106	0.828
Bivalvia	Nuculidae	*Ennucula tenuis*	SWL11	1.345	0.264	0.196	*Ennucula tenuis*	5.307	1.041
Bivalvia	Tellinidae	*Macoma calcarea*	SWL11	1.965	0.242	0.123	*Macoma calcarea*	5.385	0.663
Bivalvia	Tellinidae	*Macoma moesta*	PSEA10	0.074	0.011	0.148	*Macoma moesta*	5.113	0.758
Polychaeta	Capitellidae	*Barontolle americana*	SWL11	0.004	0.001	0.146	Capitellidae	4.860	0.711

Polychaeta	Capitellidae	*Capitella capitate*	SWL11	0.008	0.001	0.104	Capitellidae	4.860	0.505
Polychaeta	Capitellidae	*Notomastus sp.*	SWL11	0.024	0.003	0.134	Capitellidae	4.860	0.651
Polychaeta	Cirratulidae	*sp.*	PSEA10	0.132	0.019	0.142	Cirratulidae	4.897	0.694
Polychaeta	Lumbrineridae	*sp.*	PSEA10	0.043	0.008	0.188	Lumbrineridae	4.983	0.936
Polychaeta	Maldanidae	*Axiothella catenata*	PSEA10	14.929	2.736	0.183	*Axiothella catenata*	5.042	0.924
Polychaeta	Maldanidae	*Praxiella praetermisse*	SWL11	0.292	0.089	0.306	*Praxiella praetermisse*	4.995	1.530
Polychaeta	Nephtyidae	*Nephtys caeca*	SWL11	10.098	2.094	0.207	Nephtyidae	4.750	0.985
Polychaeta	Orbiniidae	*Leitoscoloplos pugattensis*	SWL11	0.084	0.013	0.152	*Leitoscoloplos pugattensis*	4.900	0.744
Polychaeta	Orbiniidae	*Scoloplos armiger*	SWL11	0.022	0.003	0.135	*Scoloplos armiger*	4.995	0.672
Polychaeta	Phyllodocidae	*Eteone longa*	PSEA10	0.001	0.000	0.231	*Eteone sp.*	4.891	1.129
Polychaeta	Phyllodocidae	*Phyllodoce groenlandica*	SWL11	0.087	0.010	0.110	Phyllodocidae	4.891	0.537
Polychaeta	Terebellidae	*Terebellides stroemi*	SWL11	0.178	0.034	0.190	Terebellidae	5.261	1.000

Figures

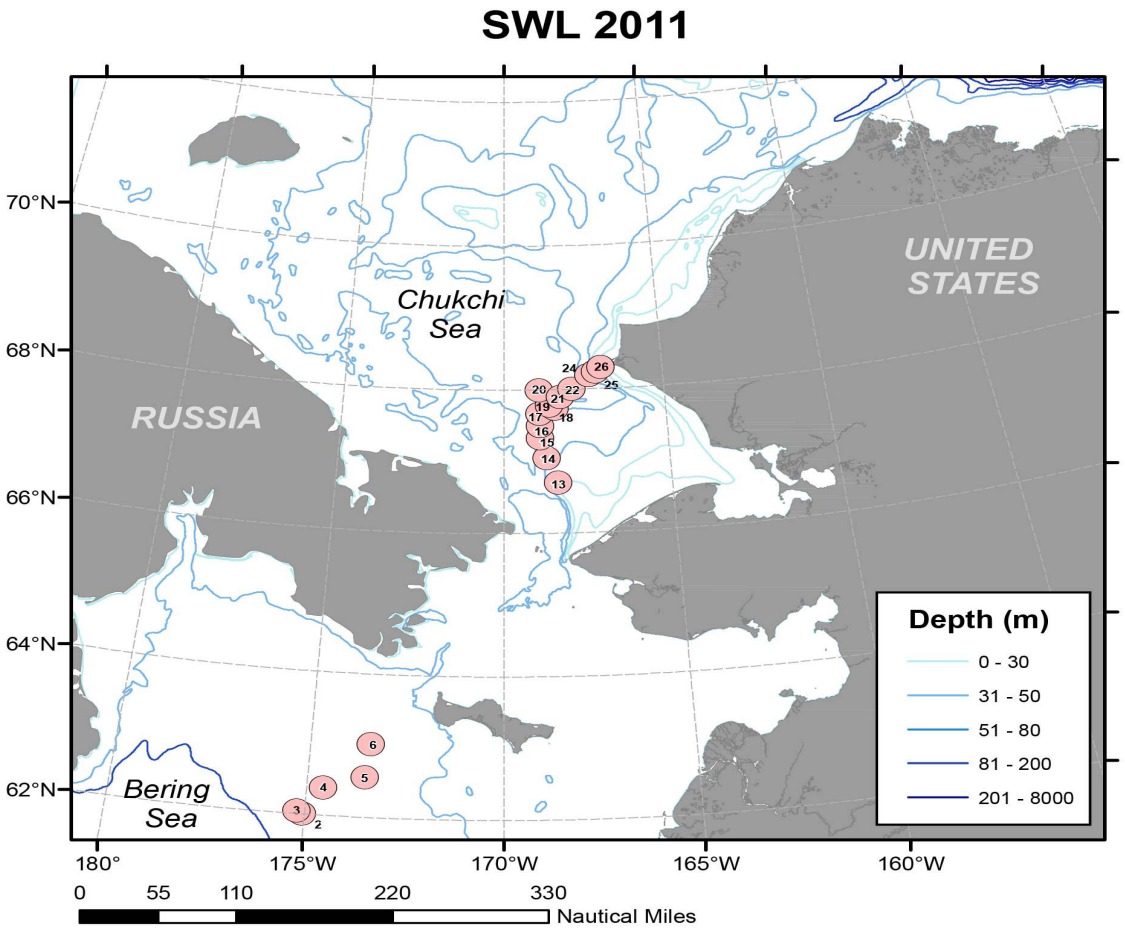

Figure 3.1: Map of the Pacific Arctic Region with study sites selected for caloric analysis during the 2011 pilot Distributed Biological Observatory (DBO) program on the July 2011 cruise of the CCGS Sir Wilfrid Laurier (SWL).

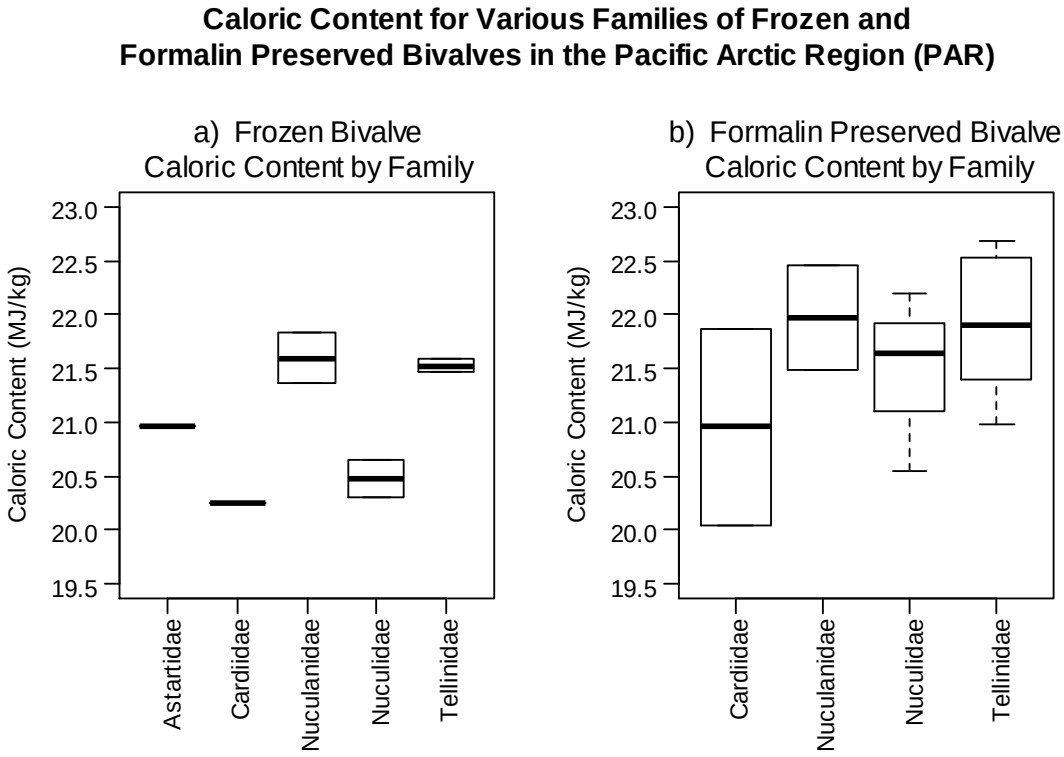

Figure 3.2: Comparison of caloric densities of bivalve families preserved frozen (a) or in formalin (b). (see Fig. 1 caption for location of stations). Animals were collected during the July 2011 CCGS Sir Wilfrid Laurier cruise. Box plots show median (horizontal line), first and third quartile (bottom and upper bounds of the box), and minimum and maximum values (whiskers).

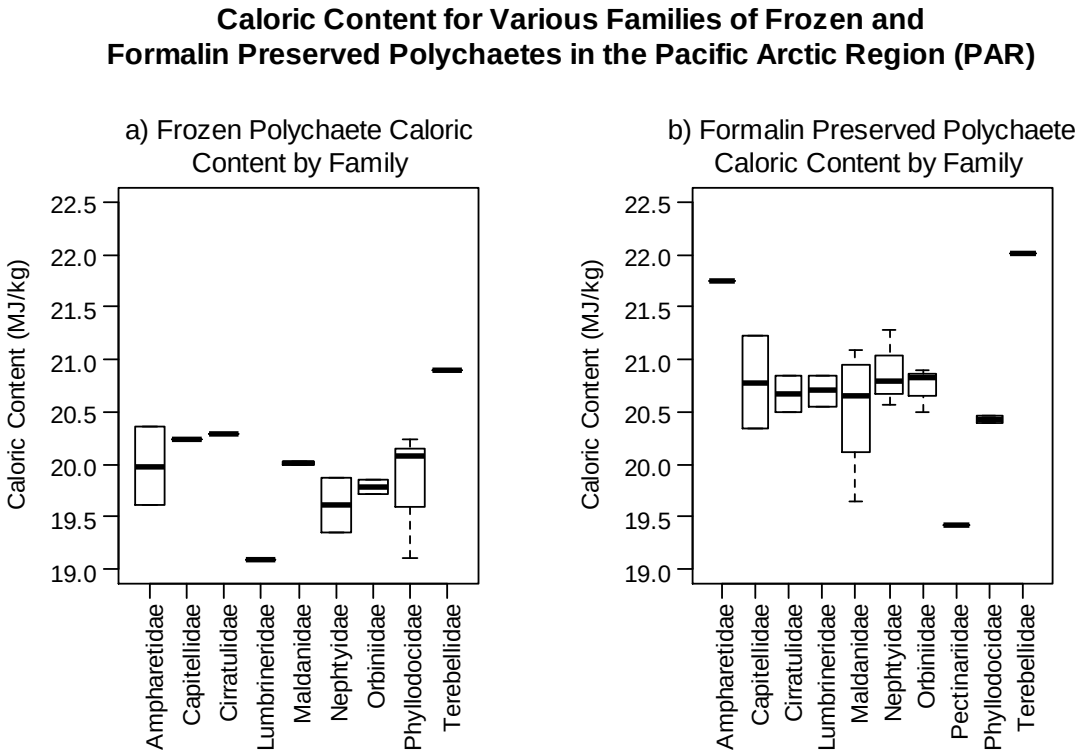

Figure 3.3: Comparison of caloric densities of polychaete families preserved (a) frozen and (b) in 10% buffered formalin (see Fig. 1 caption for station locations). Animals were collected during the July 2011 CCGS Sir Wilfrid Laurier cruise. Box plots show median (horizontal line), first and third quartile (bottom and upper bounds of the box), and minimum and maximum values (whiskers).

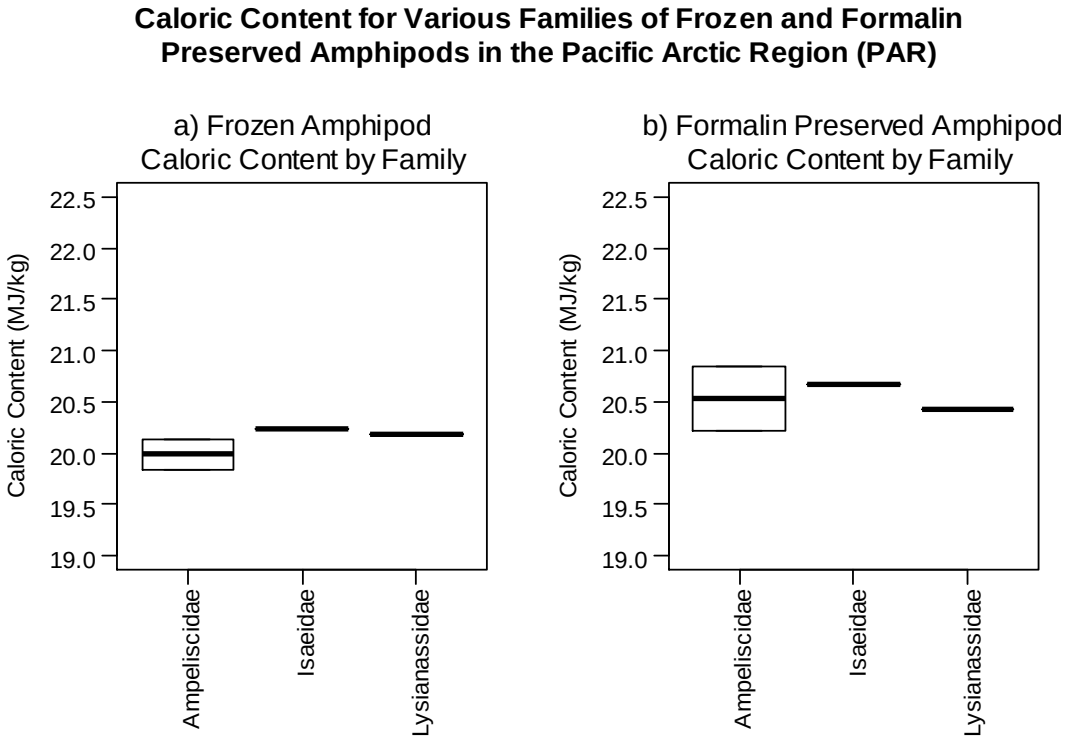

Figure 3.4: Comparison of caloric densities of three amphipod families preserved using two preservation methods: (a) frozen, and (b) in 10% buffered formalin (see Fig. 1 caption for station locations). Animals were collected during the July 2011 CCGS Sir Wilfrid Laurier cruise. Box plots show median (horizontal line), and first and third quartile (bottom and upper bounds of the box).

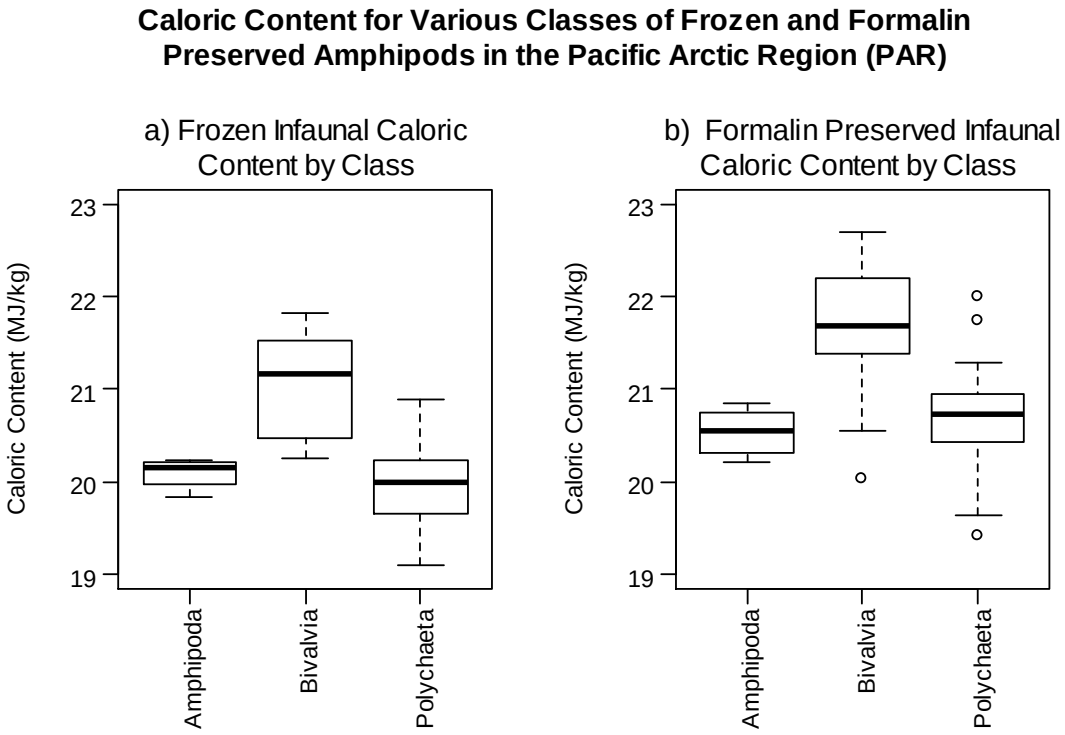

Figure 3.5: Comparison of energy densities by class for (a) frozen and (b) in 10% formalin preserved infaunal tissue samples from the Pacific Arctic Region (PAR). (see Fig. 1 caption for station locations). Animals were collected during the July 2011 CCGS Sir Wilfrid Laurier cruise. Box plots show median (horizontal line), first and third quartile (bottom and upper bounds of the box), and minimum and maximum values (whiskers). Outliers (1.5 times the inter-quartile range) are represented as open circles.

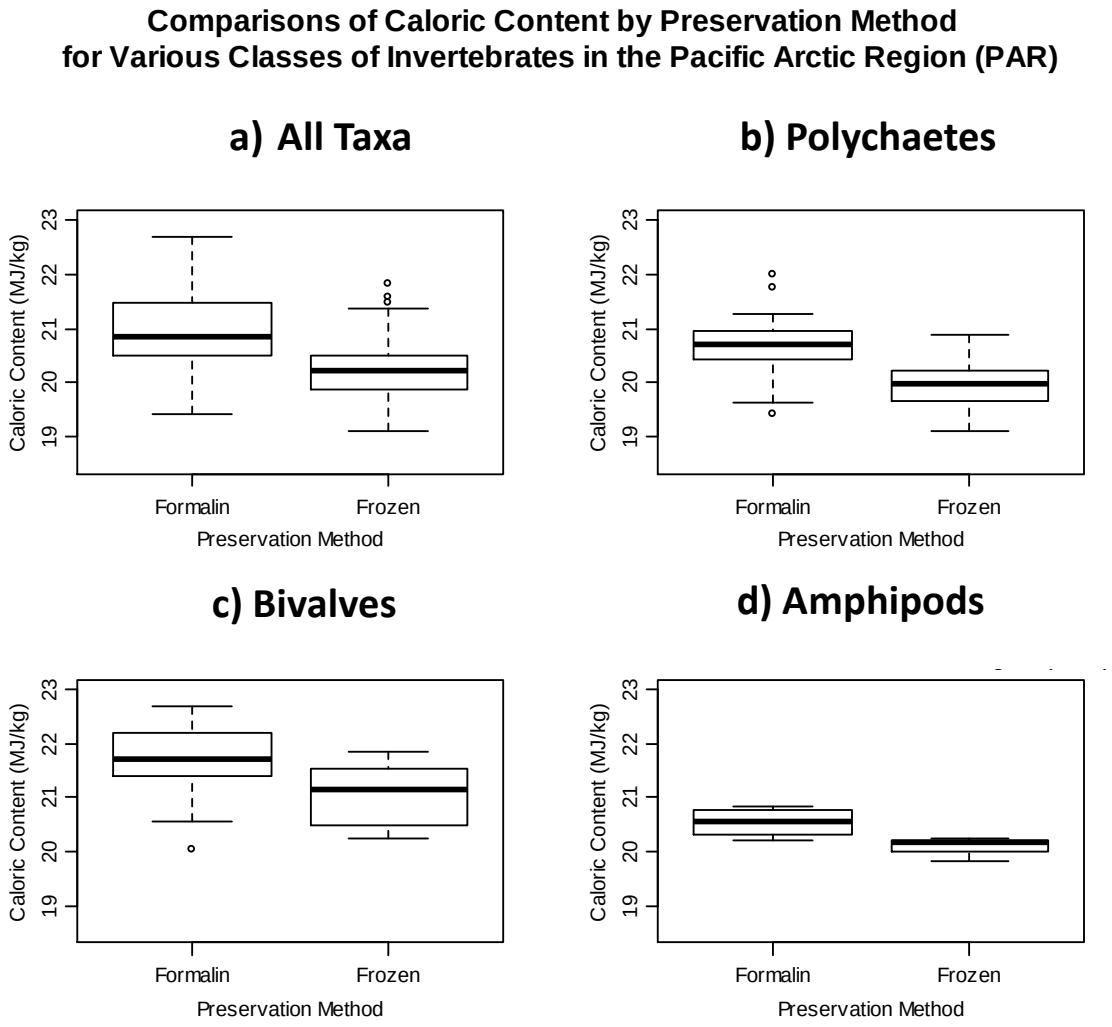

Figure 3.6: Comparison of caloric values obtained by frozen and formalin preserved preservation methods in the PAR for: (a) combined samples, (b) bivalve, (c) polychaete, and (d) amphipods. (see Fig. 1 caption for station locations). Animals were collected during the July 2011 CCGS Sir Wilfrid Laurier cruise. Box plots show median (horizontal line), first and third quartile (bottom and upper bounds of the box), and minimum and maximum values (whiskers). Outliers (1.5 times the inter-quartile range) are represented as open circles.

Chapter 4: Conclusions

The Role of Caloric Studies in Today's Arctic Research

Because of climate change and recent oil and gas interests, the Arctic has become the subject of intense research effort. The need to evaluate the current status of and long term changes in the benthic ecosystems in the region has led to a number of developing research projects, including the two with which this project was involved. The Chukchi Sea Offshore Monitoring in Drilling Area (COMIDA) Chemical and Benthos (CAB) project's goal is to develop a baseline dataset for benthic and epibenthic macroinvertebrates that dominate the Chukchi Sea, and to analyze changes in chemicals, nutrients, sediment characteristics, sedimentation rates, and trace metals (see www.comidacab.org). The goal of the new Distributed Biological Observatory (DBO) project is to integrate both benthic and water column environmental, chemical and biological observations at biological "hotspots" in the Pacific Arctic Region (PAR) and link them to higher-trophic level studies, including marine mammals and seabirds (see http://www.noaa.gov/dbo).

This caloric survey plays an important role in the goals of both of these projects. Energy studies have become a strong branch of ecology, as studying rates of energy flow allow for the direct comparison of ecosystems with different species compositions (Odum 1968). Interest in marine mammal energetics has recently grown, especially in light of environmental pressures related to climate change (Geiselman et al. 2012). Caloric studies fill an important niche in the field of bioenergetics. Determining the caloric content of prey items is necessary to understand predator food requirements (Kastelein et

al. 2000). Caloric content may also be a good proxy for high lipid content species, as zones with high caloric density are preferred feeding grounds for Arctic marine mammals (Bluhm and Gradinger 2008).

In addition to being useful for predator-prey studies, caloric studies may also be useful for evaluating benthic community health. Within any one species, typically animals with higher lipid content are considered healthier than animals with lower lipid content. For example, lipid content has been shown to be useful as an index for survival and growth in multiple bivalve species (Gallager et al. 2003), and bivalves with high levels of lipids and carbohydrate have higher fecundity than animals of similar size with lower levels of lipids and carbohydrates (Walne, 1964). Caloric surveys on benthic invertebrates in the PAR over wide spatial scales (especially with the influence of taxon regressed out) could contribute valuable information about local species health, and with continued observation, could describe changes in community health over time.

Review of Project Goals and Key Findings

In chapter 2, current caloric energy values for Chukchi Sea infaunal and epifaunal benthic macroinvertebrates were reported, and these values were analyzed for relationships to various spatial and environmental variables, including latitude, longitude, depth, bottom water temperature, bottom water salinity, sediment grain size, sediment modal size, sediment total organic carbon (TOC), sediment total organic nitrogen (TON), and sediment carbon to nitrogen ratio (C:N). From these variables, multiple models explaining caloric content in the Chukchi Sea were generated. The first two models were

generated by ANOVA, with the primary difference between them being that the first included class as an explanatory variable for caloric content, while the second had all influence on caloric content by class regressed out. The third model generated was a nested mixed effects model, with class and lowest taxon identified within class identified as random effects, while all other spatial and environmental variables were identified as fixed effects. Two approaches to partitioned (K-means and Partitioning Around Medioids (PAM)) and one hierarchical agglomerative approach to cluster analysis (Ward's method) were also conducted to assess sites with similarity in both caloric content and environmental conditions.

Throughout all of these analyses, two variables stood out as key dependencies for caloric content in the Chukchi Sea study area. The first was class, which was expected, as significant differences in high energy lipid content between classes have been demonstrated in the published literature (Parrish et al. 2009). Because class explained so much variation in energy content, it tended to obscure all other spatial and environmental variables in ANOVA. Of these variables, only latitude, being the other stand out dependency for caloric content, appeared in the best fit analysis for the linear model with taxon dependencies.

That latitude returned in both linear models and in the mixed effects model as an important explanatory variable for caloric content may reflect the physical and biological oceanography of the study area. Specifically, high nutrient Bering Shelf Anadyr Water (BSAW, also called Bering Sea water when in the Chukchi Sea) flows through the northern portion of the study area, while the low nutrient Alaska Coastal Water (ACW) flows up through the south of the study area and along the Alaskan coast. The BSAW

water mass is known to provide a higher quality carbon supply to the benthos (Grebmeier et al. 1988), resulting in higher productivity in that region than in the ACW water mass (Stoker 1978; Walsh et al. 1988). K-means cluster analysis provided further confirmation that water mass is likely responsible for the higher caloric observations in the BSAW zone, as stations in the northwest of the study area had the highest caloric density of all 6 cluster groups, and the highest surface sediment TOC and TON.

In chapter 3, a more methods-based investigation was conducted. Because caloric energy studies in the published literature have employed a number of different preservation methods, there is now a need to evaluate whether or not preservation method has a significant influence on caloric content. The main objective of chapter 3 was to compare the caloric measurements of samples preserved under two different preservation methods: freezing and formalin fixation. A secondary objective was to generate conversion values between wet infaunal weight and energy (calories) for multiple taxa, allowing for the "unlocking" of many years of biomass data for energetics.

While the results of chapter 3 once again confirmed that significant differences in caloric content between taxa exist, the key result of chapter 3 was that a significant difference in caloric content was measured between formalin fixed and frozen tissues. In almost every case, formalin fixed tissues measured at higher caloric densities than frozen tissues, highlighting the need to take preservation method into account when conducting or cross-comparing caloric studies. One important consideration, however, is whether or not the difference is large enough to have an impact on benthic studies. The increase due to formalin was 3.3%, which in a small scale study may not have any large impact, but in a larger spatial scale study, perhaps the difference would be important.

Future Work

Connecting caloric surveys to higher trophic-level predators is a goal for future research. The conversion factors generated in chapter 3 make it possible to convert the dominant infaunal benthic biomass data in the PAR to caloric content. This process would be useful for evaluating the health of benthic infaunal assemblages in the PAR over wide temporal and spatial scales, and may be very informative in predator-prey studies. That caloric content increases significantly from south to north through the Chukchi Sea study area may have direct implications for Pacific walrus as they adapt to decreases in seasonal sea ice cover as traditional habitat (Jay et al. 2011). Quantitatively connecting spatial variation in caloric content with the altered foraging behavior of walruses will be the next step toward evaluating outlook for the species in the years to come.

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Appendices

Appendix 1: Listing and description of all R packages used in this project, ordered by package name.

R Package Name	Version	Description	Publication Date	Authors and Contributors
akima	0.5-7	Linear or cubic spline interpolation for irregular gridded data.	1/8/2008	Akima H, Gebhardt A, Petzoldt T, Maechler M
base	2.15.0	R base package.	3/31/2012	R Development Core Team and Contributors
boot	1.3-4	Functions and datasets for bootstrapping.	3/12/2012	Canty A, Ripley B
car	2.0-12	Companion to Applied Regression.	1/17/2012	Fox J, Weisberg S, Bates D, Firth D, Friendly M, Gorjanc G, Graves S, Heiberger R, Laboissiere R, Monette G, Nilsson H, Ogle D, Ripley B, Zeileis A
class	7.3-3	Various functions for classification.	12/9/2010	Ripley B
cluster	1.14.2	Cluster Analysis.	2/8/2012	Maechler M, Rousseeuw P, Struyf A, Hubert M, Hornik K
codetools	0.2-8	Code analysis tools for R.	2/15/2011	Tierney L
colorspace	1.1-1	Carries out mapping between assorted color spaces.	1/13/2012	Ihaka R, Murrell P, Hornik K, Zeileis A
compiler	2.15.0	R base package.	3/31/2012	R Development Core Team and Contributors
corrgram	1.2	Calculates correlation of variables and displays the results graphically.	3/28/2012	Wright K
datasets	2.15.0	R base package.	3/31/2012	R Development Core Team and Contributors
fields	6.6.3	Companion for spatial prediction.	1/3/2012	Furrer R, Nychka D, Sain S
flexmix	2.3-8	Implements a general framework for finite mixtures of regression models using the EM algorithm.	5/9/2012	Leisch F, Gruen B
foreign	0.8-50	Functions for reading and writing data stored by statistical packages.	5/23/2012	R Development Core Team and Contributors, Bivand R, Carey VJ, DebRoy S, Eglen S, Guha R, Lewin-Koh N, Myatt M, Pfaff B, Warmerdam F, Weigand S, Free Software Foundation, Inc.
fpc	2.0-3	Various methods for clustering and cluster validation.	11/19/2010	Henning C

gclus	1.3	Orders panels in scatterplot matrices and parallel coordinate displays by some merit index.	5/25/2010	Hurley C
graphics	2.15.0	R base package.	3/31/2012	R Development Core Team and Contributors
grDevices	2.15.0	R base package.	3/31/2012	R Development Core Team and Contributors
grid	2.15.0	R base package.	3/31/2012	R Development Core Team and Contributors
Hmisc	3.9-3	Includes support for high-level graphics, utility operations, functions for computing sample size and power, importing datasets, imputing missing values, advanced table making, variable clustering.	3/29/2012	Harrel FE Jr, Contributors
KernSmooth	2.23-7	Kernel smoothing package.	11/9/2011	Wand M, Ripley B
languageR	1.4	Data sets exemplifying statistical methods, and some facilitatory utility functions.	12/30/2011	Baayen RH
lattice	0.20-6	Data visualization system.	3/10/2012	Sarkar D
latticeExtra	0.6-19	Extra graphical utilities based on lattice package.	10/20/2011	Sarkar D, Andrews F
leaps	2.9	Regression subset selection including exhaustive search.	5/5/2009	Lumley T, Miller A
lme4	0.999375-42	Fits linear and generalized linear mixed-effects models.	10/4/2011	Bates D, Maechler M, Bolker B
mapdata	2.2-1	Supplement to maps package, providing the larger and/or higher-resolution databases.	1/13/2012	Becker RA, Wilks AR, Brownrigg R
maps	2.2-6	Displays maps with the support of other packages.	5/15/2012	Becker RA, Wilks AR, Brownrigg R, Minka TP
MASS	7.3-18	Additional function and dataset support.	5/28/2012	Ripely B, Hornik K, Gebhardt A, Firth D
Matrix	1.0-6	Creates matrices.	3/30/2012	Bates D, Maechler M
mclust	3.4.11	Model-based clustering and normal mixture modeling including Bayesian regularization.	1/7/2012	Fraley C, Raftery A
methods	2.15.0	R base package.	3/31/2012	R Development Core Team and Contributors
mgcv	1.7-16	Routines for GAMs and other generalized ridge regression with multiple smoothing parameter selection by GCV, REML or UBRE/AIC. Also GAMMs. Includes a gam() function.	6/12/2012	Wood S
modeltools	0.2-19	A collection of tools to deal with statistical models.	1/31/2012	Hothorn T, Leisch F, Zeileis A
multcomp	1.2-12	Simultaneous tests and confidence intervals for general linear hypotheses in	3/9/2012	Hothorn T, Bretz F, Westfal P

		parametric models, including linear, generalized linear, linear mixed effects, and survival models.		
mvtnorm	0.9-9992	Computes multivariate normal and t probabilities, quantiles, random deviates and densities.	1/20/2012	Genz A, Bretz F, Miwa T, Mi X, Leisch F, Scheipl F, Bornkamp B, Hothorn T
nlme	3.1-104	Fits and compares Gaussian linear and nonlinear mixed-effects models.	5/23/2012	Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team
nnet	7.3-1	Software for feed-forward neural networks with a single hidden layer, and for multinomial log-linear models.	10/28/2009	Ripley B
nortest	1.0-1	Five omnibus tests for the composite hypothesis of normality.	4/24/2012	Gross J, Ligges U
parallel	2.15.0	R base package.	3/31/2012	R Development Core Team and Contributors
plyr	1.7.1	Compartmentalizer and synthesizer.	1/8/2012	Wickham H
pspearman	0.2-5	Spearman's rank correlation test with precomputed exact null distribution for n <= 22.	6/19/2009	Savicky P
pvclust	1.2-2	Assesses the uncertainty in hierarchical cluster analysis.	4/13/2011	Suzuki R, Shimodaira H
RColorBrewer	1.0-5	Provides palettes for drawing maps shaded according to a variable.	6/17/2011	Neuwirth E
rpart	3.1-52	Recursive partitioning and regression trees.	3/4/2012	Therneu TM, Atkinson B, Ripley B
seriation	1.0-6	Infrastructure for seriation with an implementation of several seriation/sequencing.techniques to reorder matrices, dissimilarity matrices, and dendrograms.	10/19/2011	Hahsler M, Buchta C, Hornik K
spam	0.29-1	Set of function for sparse matrix algebra.	5/5/2012	Furrer R
spatial	7.3-3	Computes analysis of variance tables for one or more fitted trend surface model objects.	3/5/2011	Ripley B
splines	2.15.0	R base package.	3/31/2012	R Development Core Team and Contributors
stats	2.15.0	R base package.	3/31/2012	R Development Core Team and Contributors
stats4	2.15.0	R base package.	3/31/2012	R Development Core Team and Contributors
SuppDists	1.1-8	Ten distributions supplementing those built into R. Inverse Gauss, Kruskal-Wallis, Kendall's Tau, Friedman's chi squared,	12/11/2009	Wheeler B

		Spearman's rho, maximum F ratio, the Pearson product moment correlation coefficiant, Johnson distributions, normal scores and generalized hypergeometric distributions.		
survival	2.36-14	Descriptive statistics, two-sample tests, parametric accelerated failure models, Cox model.	4/25/2012	Therneu T, Lumley T
tcltk	2.15.0	R base package.	3/31/2012	R Development Core Team and Contributors
tools	2.15.0	R base package.	3/31/2012	R Development Core Team and Contributors
TSP	1.0-6	Basic infrastructure and some algorithms.	11/29/2011	Hahsler M, Hornik K
utils	2.15.0	R base package.	3/31/2012	R Development Core Team and Contributors

Appendix 2: Measured caloric densities (MJ/kg) for all frozen benthic and epibenthic macroinvertebrates collected during the July-August 2010 survey of the Chukchi Sea as part of the Chukchi Sea Offshore Monitoring in Drilling Area (COMIDA) Chemical and Benthos (CAB) project. Ordered by station number, class, and lowest taxon identified.

Station Number	Station Name	Offshore Zone	Latitude (°N)	Longitude (°W)	Class	Lowest Taxon Identified	Caloric Content (MJ/kg)
4	RDM	Nearshore	67.562	-164.178	Gastropoda	Buccinidae	21.096
4	RDM	Nearshore	67.562	-164.178	Gastropoda	Buccinidae	21.288
4	RDM	Nearshore	67.562	-164.178	Malacostraca	Oregoniidae	14.946
4	RDM	Nearshore	67.562	-164.178	Malacostraca	Paguridae	17.276
4	RDM	Nearshore	67.562	-164.178	Polychaeta	Maldanidae	18.997
4	RDM	Nearshore	67.562	-164.178	Sipunculidea	Golfingiidae	16.251
6	CBL1	Nearshore	69.040	-166.594	Asteroidea	Asteroidea	15.072
6	CBL1	Nearshore	69.040	-166.594	Gastropoda	Buccinidae	21.824
6	CBL1	Nearshore	69.040	-166.594	Gastropoda	Buccinidae	22.383
6	CBL1	Nearshore	69.040	-166.594	Gastropoda	Naticidae	20.271
6	CBL1	Nearshore	69.040	-166.594	Malacostraca	Oregoniidae	15.280
6	CBL1	Nearshore	69.040	-166.594	Polychaeta	Maldanidae	20.242
6	CBL1	Nearshore	69.040	-166.594	Sipunculidea	Golfingiidae	16.300
8	107	Nearshore	70.086	-166.455	Ascidiacea	Boltenia	15.174
8	107	Nearshore	70.086	-166.455	Bivalvia	Astartidae	21.151
8	107	Nearshore	70.086	-166.455	Bivalvia	Nuculanidae	23.226
8	107	Nearshore	70.086	-166.455	Gastropoda	Buccinidae	20.773
8	107	Nearshore	70.086	-166.455	Gastropoda	Buccinidae	21.185
8	107	Nearshore	70.086	-166.455	Malacostraca	Oregoniidae	15.668
8	107	Nearshore	70.086	-166.455	Polychaeta	Maldanidae	20.682
8	107	Nearshore	70.086	-166.455	Sipunculidea	Golfingiidae	19.984
10	CBL5	Nearshore	70.023	-163.761	Ascidiacea	Mogulidae	16.798
10	CBL5	Nearshore	70.023	-163.761	Asteroidea	Asteroidea	17.573
10	CBL5	Nearshore	70.023	-163.761	Bivalvia	Veneridae	20.769
10	CBL5	Nearshore	70.023	-163.761	Echinoidea	Echinoidea	15.133
10	CBL5	Nearshore	70.023	-163.761	Malacostraca	Mysidacea	17.397
10	CBL5	Nearshore	70.023	-163.761	Malacostraca	Paguridae	17.210
16	CBL4	Midshore	70.831	-167.787	Amphipoda	Lysianassidae	19.476
16	CBL4	Midshore	70.831	-167.787	Anthozoa	Anemone	20.063
16	CBL4	Midshore	70.831	-167.787	Asteroidea	Asteroidea	16.427
16	CBL4	Midshore	70.831	-167.787	Bivalvia	Astartidae	17.595
16	CBL4	Midshore	70.831	-167.787	Bivalvia	Cardiidae	20.218
16	CBL4	Midshore	70.831	-167.787	Bivalvia	Nuculidae	21.030
16	CBL4	Midshore	70.831	-167.787	Bivalvia	Nuculidae	21.423
16	CBL4	Midshore	70.831	-167.787	Bivalvia	Tellinidae	20.024
16	CBL4	Midshore	70.831	-167.787	Bivalvia	Yoldiidae	21.765

16	CBL4	Midshore	70.831	-167.787	Gastropoda	Buccinidae	20.487
16	CBL4	Midshore	70.831	-167.787	Gastropoda	Buccinidae	21.376
16	CBL4	Midshore	70.831	-167.787	Gastropoda	Naticidae	18.928
16	CBL4	Midshore	70.831	-167.787	Malacostraca	Oregoniidae	16.063
16	CBL4	Midshore	70.831	-167.787	Malacostraca	Paguridae	17.724
18	UTX16	Midshore	71.249	-165.448	Asteroidea	Asteroidea	15.152
18	UTX16	Midshore	71.249	-165.448	Bivalvia	Astartidae	21.416
18	UTX16	Midshore	71.249	-165.448	Bivalvia	Nuculanidae	22.063
18	UTX16	Midshore	71.249	-165.448	Bivalvia	Nuculidae	22.001
18	UTX16	Midshore	71.249	-165.448	Bivalvia	Tellinidae	21.873
18	UTX16	Midshore	71.249	-165.448	Gastropoda	Buccinidae	21.545
18	UTX16	Midshore	71.249	-165.448	Gastropoda	Buccinidae	21.549
18	UTX16	Midshore	71.249	-165.448	Gastropoda	Naticidae	20.976
18	UTX16	Midshore	71.249	-165.448	Malacostraca	Mysidacea	21.606
18	UTX16	Midshore	71.249	-165.448	Malacostraca	Oregoniidae	17.644
18	UTX16	Midshore	71.249	-165.448	Malacostraca	Paguridae	17.899
20	1014	Nearshore	70.840	-163.291	Anthozoa	Anthozoa	16.679
20	1014	Nearshore	70.840	-163.291	Ascidiacea	Boltenia	16.040
20	1014	Nearshore	70.840	-163.291	Asteroidea	Asteroidea	16.862
20	1014	Nearshore	70.840	-163.291	Bivalvia	Astartidae	22.251
20	1014	Nearshore	70.840	-163.291	Gastropoda	Buccinidae	19.503
20	1014	Nearshore	70.840	-163.291	Gastropoda	Buccinidae	20.244
20	1014	Nearshore	70.840	-163.291	Gastropoda	Naticidae	22.232
20	1014	Nearshore	70.840	-163.291	Gastropoda	Trochidae	21.441
20	1014	Nearshore	70.840	-163.291	Holothuroidea	Holothuroidea	16.387
20	1014	Nearshore	70.840	-163.291	Malacostraca	Mysidacea	21.437
20	1014	Nearshore	70.840	-163.291	Malacostraca	Oregoniidae	18.830
20	1014	Nearshore	70.840	-163.291	Malacostraca	Paguridae	17.439
20	1014	Nearshore	70.840	-163.291	Polychaeta	Maldanidae	20.542
21	CBL16	Nearshore	71.414	-157.491	Ascidiacea	Styelidae	17.485
21	CBL16	Nearshore	71.414	-157.491	Bivalvia	Astartidae	19.947
21	CBL16	Nearshore	71.414	-157.491	Bivalvia	Mytilidae	19.642
21	CBL16	Nearshore	71.414	-157.491	Bivalvia	Tellinidae	21.459
21	CBL16	Nearshore	71.414	-157.491	Gastropoda	Buccinidae	19.648
21	CBL16	Nearshore	71.414	-157.491	Sipunculidea	Golfingiidae	18.463
24	CBL15	Midshore	71.727	-160.718	Anthozoa	Anthozoa	19.125
24	CBL15	Midshore	71.727	-160.718	Asteroidea	Asteroidea	17.069
24	CBL15	Midshore	71.727	-160.718	Bivalvia	Astartidae	21.213
24	CBL15	Midshore	71.727	-160.718	Bivalvia	Carditidae	21.878
24	CBL15	Midshore	71.727	-160.718	Bivalvia	Mytilidae	21.421
24	CBL15	Midshore	71.727	-160.718	Bivalvia	Nuculanidae	21.868
24	CBL15	Midshore	71.727	-160.718	Bivalvia	Nuculidae	20.828
24	CBL15	Midshore	71.727	-160.718	Bivalvia	Tellinidae	21.619

24	CBL15	Midshore	71.727	-160.718	Gastropoda	Buccinidae	21.110
24	CBL15	Midshore	71.727	-160.718	Gastropoda	Naticidae	21.814
24	CBL15	Midshore	71.727	-160.718	Gastropoda	Neptunea	21.257
24	CBL15	Midshore	71.727	-160.718	Malacostraca	Idoteidae	17.719
24	CBL15	Midshore	71.727	-160.718	Malacostraca	Oregoniidae	18.697
24	CBL15	Midshore	71.727	-160.718	Malacostraca	Paguridae	17.917
24	CBL15	Midshore	71.727	-160.718	Polychaeta	Maldanidae	20.337
24	CBL15	Midshore	71.727	-160.718	Polychaeta	Onuphidae	20.596
27	HSH1	Midshore	72.101	-162.975	Ascidiacea	Styelidae	13.698
27	HSH1	Midshore	72.101	-162.975	Bivalvia	Astartidae	20.908
27	HSH1	Midshore	72.101	-162.975	Bivalvia	Tellinidae	22.757
27	HSH1	Midshore	72.101	-162.975	Gastropoda	Buccinidae	20.717
27	HSH1	Midshore	72.101	-162.975	Gastropoda	Buccinidae	21.318
27	HSH1	Midshore	72.101	-162.975	Gastropoda	Naticidae	21.031
27	HSH1	Midshore	72.101	-162.975	Malacostraca	Mysidacea	23.244
27	HSH1	Midshore	72.101	-162.975	Malacostraca	Oregoniidae	18.807
27	HSH1	Midshore	72.101	-162.975	Malacostraca	Paguridae	17.877
27	HSH1	Midshore	72.101	-162.975	Sipunculidea	Golfingiidae	21.469
30	UTX11	Midshore	71.453	-162.611	Ascidiacea	Styelidae	17.481
30	UTX11	Midshore	71.453	-162.611	Asteroidea	Asteroidea	19.928
30	UTX11	Midshore	71.453	-162.611	Bivalvia	Astartidae	21.037
30	UTX11	Midshore	71.453	-162.611	Bivalvia	Mytilidae	21.324
30	UTX11	Midshore	71.453	-162.611	Bivalvia	Nuculidae	20.670
30	UTX11	Midshore	71.453	-162.611	Bivalvia	Tellinidae	20.778
30	UTX11	Midshore	71.453	-162.611	Bivalvia	Veneridae	20.048
30	UTX11	Midshore	71.453	-162.611	Gastropoda	Naticidae	20.586
30	UTX11	Midshore	71.453	-162.611	Gastropoda	Neptunea	20.436
30	UTX11	Midshore	71.453	-162.611	Gastropoda	Nudibranch	20.143
30	UTX11	Midshore	71.453	-162.611	Gastropoda	Trochidae	20.535
30	UTX11	Midshore	71.453	-162.611	Holothuroidea	Psolidae	15.792
30	UTX11	Midshore	71.453	-162.611	Malacostraca	Asteroidea	19.373
30	UTX11	Midshore	71.453	-162.611	Malacostraca	Oregoniidae	17.848
30	UTX11	Midshore	71.453	-162.611	Malacostraca	Paguridae	17.255
30	UTX11	Midshore	71.453	-162.611	Polychaeta	Maldanidae	19.211
30	UTX11	Midshore	71.453	-162.611	Polychaeta	Phyllodocidae	20.245
30	UTX11	Midshore	71.453	-162.611	Polychaeta	Polynoidae	19.772
32	UTX5	Midshore	71.702	-164.515	Asteroidea	Asteroidea	17.413
32	UTX5	Midshore	71.702	-164.515	Bivalvia	Nuculidae	20.231
32	UTX5	Midshore	71.702	-164.515	Bivalvia	Tellinidae	22.560
32	UTX5	Midshore	71.702	-164.515	Gastropoda	Buccinidae	21.137
32	UTX5	Midshore	71.702	-164.515	Gastropoda	Naticidae	20.750
32	UTX5	Midshore	71.702	-164.515	Gastropoda	Neptunea	20.952
32	UTX5	Midshore	71.702	-164.515	Malacostraca	Mysidacea	19.625

32	UTX5	Midshore	71.702	-164.515	Malacostraca	Oregoniidae	20.255
32	UTX5	Midshore	71.702	-164.515	Malacostraca	Paguridae	18.190
32	UTX5	Midshore	71.702	-164.515	Polychaeta	Lumbrineridae	20.520
32	UTX5	Midshore	71.702	-164.515	Polychaeta	Maldanidae	21.201
32	UTX5	Midshore	71.702	-164.515	Sipunculidea	Golfingiidae	18.045
34	1030	Offshore	72.103	-165.456	Anthozoa	Anthozoa	18.514
34	1030	Offshore	72.103	-165.456	Asteroidea	Asteroidea	14.240
34	1030	Offshore	72.103	-165.456	Bivalvia	Astartidae	20.090
34	1030	Offshore	72.103	-165.456	Bivalvia	Cardiidae	20.701
34	1030	Offshore	72.103	-165.456	Bivalvia	Carditidae	22.038
34	1030	Offshore	72.103	-165.456	Bivalvia	Nuculanidae	17.905
34	1030	Offshore	72.103	-165.456	Bivalvia	Nuculidae	17.630
34	1030	Offshore	72.103	-165.456	Bivalvia	Tellinidae	18.796
34	1030	Offshore	72.103	-165.456	Bivalvia	Yoldiidae	20.838
34	1030	Offshore	72.103	-165.456	Gastropoda	Buccinidae	19.653
34	1030	Offshore	72.103	-165.456	Gastropoda	Buccinidae	20.121
34	1030	Offshore	72.103	-165.456	Gastropoda	Buccinidae	21.287
34	1030	Offshore	72.103	-165.456	Gastropoda	Muricidae	19.745
34	1030	Offshore	72.103	-165.456	Gastropoda	Naticidae	20.638
34	1030	Offshore	72.103	-165.456	Malacostraca	Mysidacea	22.293
34	1030	Offshore	72.103	-165.456	Malacostraca	Oregoniidae	17.671
34	1030	Offshore	72.103	-165.456	Malacostraca	Paguridae	17.722
34	1030	Offshore	72.103	-165.456	Polychaeta	Nephtyidae	20.245
36	UTX3	Offshore	71.930	-167.389	Asteroidea	Asteroidea	18.414
36	UTX3	Offshore	71.930	-167.389	Bivalvia	Carditidae	21.730
36	UTX3	Offshore	71.930	-167.389	Bivalvia	Nuculidae	20.828
36	UTX3	Offshore	71.930	-167.389	Bivalvia	Tellinidae	22.673
36	UTX3	Offshore	71.930	-167.389	Bivalvia	Yoldiidae	21.567
36	UTX3	Offshore	71.930	-167.389	Gastropoda	Buccinidae	21.145
36	UTX3	Offshore	71.930	-167.389	Gastropoda	Naticidae	21.035
36	UTX3	Offshore	71.930	-167.389	Gastropoda	Neptunea	21.156
36	UTX3	Offshore	71.930	-167.389	Malacostraca	Mysidacea	19.679
36	UTX3	Offshore	71.930	-167.389	Malacostraca	Oregoniidae	19.059
36	UTX3	Offshore	71.930	-167.389	Malacostraca	Paguridae	20.890
36	UTX3	Offshore	71.930	-167.389	Polychaeta	Maldanidae	21.608
36	UTX3	Offshore	71.930	-167.389	Polychaeta	Nephtyidae	21.862
38	CBL8	Offshore	71.485	-167.782	Anthozoa	Anthozoa	18.227
38	CBL8	Offshore	71.485	-167.782	Asteroidea	Asteroidea	15.824
38	CBL8	Offshore	71.485	-167.782	Bivalvia	Astartidae	18.898
38	CBL8	Offshore	71.485	-167.782	Bivalvia	Carditidae	21.129
38	CBL8	Offshore	71.485	-167.782	Bivalvia	Nuculanidae	23.494
38	CBL8	Offshore	71.485	-167.782	Bivalvia	Nuculidae	22.430
38	CBL8	Offshore	71.485	-167.782	Bivalvia	Tellinidae	21.735

38	CBL8	Offshore	71.485	-167.782	Gastropoda	Buccinidae	21.241
38	CBL8	Offshore	71.485	-167.782	Gastropoda	Naticidae	20.208
38	CBL8	Offshore	71.485	-167.782	Malacostraca	Mysidacea	19.546
38	CBL8	Offshore	71.485	-167.782	Malacostraca	Oregoniidae	17.284
38	CBL8	Offshore	71.485	-167.782	Malacostraca	Paguridae	20.154
38	CBL8	Offshore	71.485	-167.782	Polychaeta	Maldanidae	21.342

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